НАЦІОНАЛЬНА АКАДЕМІЯ НАУК УКРАЇНИ
ІНСТИТУТ ОРГАНІЧНОЇ ХІМІЇ НАН УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

Рік заснування – 1966

ЖУРНАЛ ОРГАНІЧНОЇ ТА ФАРМАЦЕВТИЧНОЇ ХІМІЇ

JOURNAL OF ORGANIC AND PHARMACEUTICAL CHEMISTRY

2023 — том 21, випуск 4 (84)

Харків
НФаУ

У журналі розглянуто проблеми синтезу й аналізу органічних та елементоорганічних сполук, аналогів природних сполук і лікарських субстанцій, наведено результати фізико-хімічних досліджень у вищезазначених напрямах. Також з погляду (біо)органічної, фармацевтичної, аналітичної та фізичної хімії проаналізовано питання з різних аспектів рослинництва, ґрунтознавства й дослідження навколишнього середовища.

Для працівників науково-дослідних установ, вищих навчальних закладів та фахівців хімічного, фармацевтичного, біологічного, медичного і сільськогосподарського профілів.

«Журнал органічної та фармацевтичної хімії» внесено до затвердженого МОН України Переліку наукових фахових видань України (категорія «Б») для опублікування результатів дисертаційних робіт за спеціальністю 102 – Хімія та 226 – Фармація, промислова фармація (наказ МОН України від 28.12.2019 р. № 1643); індексовано в наукометричних базах даних: Chemical Abstracts (CAS), Index Copernicus; внесено до каталогів та пошукових систем: Directory of Open Access Journals (DOAJ), Bielefeld Academic Search Engine (BASE), Directory of Open Access scholarly Resources (ROAD), PKP Index, Ulrich's periodicals, Worldcat, НБУ ім. В. І. Вернадського і УРЖ «Джерело».

Затверджено до друку вченою радою Інституту органічної хімії НАН України, протокол № 25 від 22.12.2023 р.

Затверджено до друку вченою радою Національного фармацевтичного університету, протокол № 11 від 11.12.2023 р.

Адреса для листування: 61002, м. Харків, вул. Пушкінська, 53, Національний фармацевтичний університет, редакція «Журналу органічної та фармацевтичної хімії». E-mail: publish@nuph.edu.ua, orgpharm-journal@nuph.edu.ua. Сайт: http://ophcj.nuph.edu.ua

Передплатні індекси: для індивідуальних передплатників — 08383, для підприємств — 08384

Свідоцтво про державну реєстрацію серії КВ № 23086-12926ПР від 05.01.2018 р.

Підписано до друку 25.12.2023 р. Формат 60 × 84 1/8.

Папір офсетний. Друк ризо. Умовн. друк. арк. 9,3. Обліков.-вид. арк. 10,76. Тираж 50 прим.

Редактори — О. Ю. Гурко, Л. І. Дубовик. Комп'ютерне верстання — О. М. Білинська

«Журнал органічної та фармацевтичної хімії». Том 21, випуск 4 (84), 2023

ISSN 2308-8303 (Print)

ISSN 2518-1548 (Online)

Journal of Organic and Pharmaceutical Chemistry

Original Research

OPEN ACCESS
Freely available online on
http://ophcj.nuph.edu.ua

UDC 547.814.1+ 547.789.1 + 544.174

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Synthesis of a New Series of Chromones Based on Formylthiazoles

Abstract
A preparative approach to thiazole-containing chromone derivatives has been developed by modifying the corresponding aldehydes with their further transformation into propenone derivatives, and finally introducing them into the Algar-Flynn-Oyamada reaction. Several methods for obtaining propenones have been analyzed, and the most effective and practically convenient one has been found. The thiazole-containing analogs of chromones obtained have a great potential as probes for a wide range of studies.
Keywords: thiazole; chromone; heterocycle; complex formation

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Синтез нового ряду хромонів на основі формілтіазолів

Анотація
Розроблено препаративний підхід до тіазолвмісних похідних хромону шляхом модифікації відповідних альдегідів та наступним одержанням на їх основі похідних пропенонів, які було застосовано в реакції Альгара-Флінна-Оямади. Проаналізовано декілька методів одержання пропенонів та виявлено найбільш ефективний та практично зручний. Отримані тіазолвмісні аналоги хромонів мають великий потенціал як зонди для широкого спектра досліджень.
Ключові слова: тіазол; хромон; гетероцикл; комплексоутворення

Citation: Tarasenko, D. O.; Chumak, A. Y.; Kolomoitsev, O. O.; Kotliar, V. M.; Roshal, A. D. The synthesis of a new series of chromones based on formylthiazoles. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 3–10.
https://doi.org/10.24959/ophcj.23.292844
Supporting information: Copies of ^{1}H and ^{13}C NMR spectra of the synthesized compounds.
Received: *5 October 2023*; **Revised:** *17 November 2023*; **Accepted:** *25 November 2023*

Funding: The authors received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

Various flavonols are currently widely used primarily due to their luminescent properties and sensitivity to various parameters of media [1, 2]. They can be used both as detectors for some cations [3, 4] and anions [5], as well as for individual neutral compounds [6, 7]. They are also useful in studying the properties of the medium [8–11] and biochemical studies [12–14], some of them are also used as probes to study drug delivery to the required places in "containers" [15].

However, the potential for application of flavonols is much wider. And their properties are currently being studied [16–18]; the results of these studies may further expand the scope of flavonols.

Therefore, although the methods for the synthesis of many flavonols and other chromones have been known for a long time [19, 20], researching new ways for obtaining chromones remains an important issue. Their heterocyclic analogs are especially interesting in this regard since they create several additional centers for forming complexes, as well as due to the heterocyclic substituent effect on the electronic transition.

Developments in the field of heterocyclic analogs of flavonols – the synthesis of hetarylchromones – are particularly interesting and relevant

as they are promising complexing agents. Moreover, hetarylchromones may have interesting spectral properties due to the effect of the heterocyclic fragment on the electronic structure of molecules.

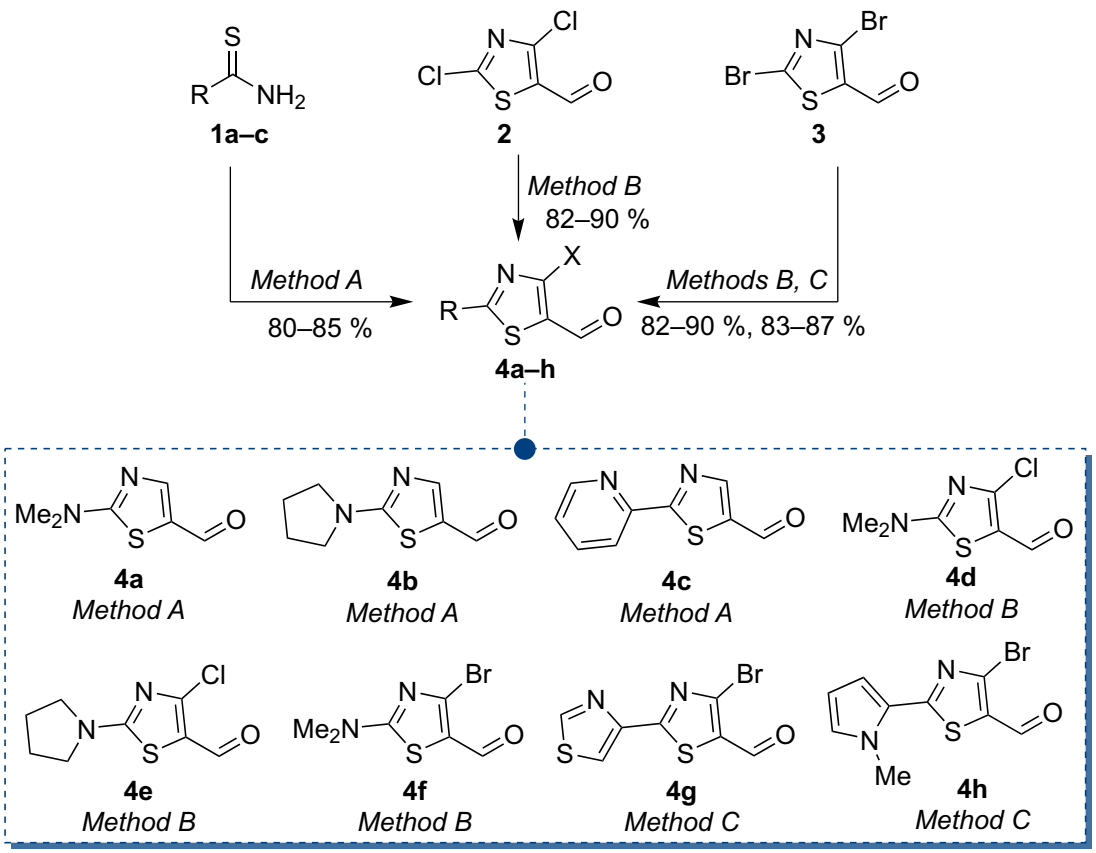
In this work, we present a synthetic approach that allows synthesizing a new series of thiazole-containing chromones.

■ Results and discussion

Chromones were planned to be obtained by the Algar-Flynn-Oyamada reaction, starting from the corresponding analogs of chalcones. The latter were prepared by the Claisen condensation of the corresponding aldehydes with 2-hydroxyacetophenone. Thus, initially 5-formylthiazoles **4a–c** with dimethylamino, 1-pyrrolidinyl and 2-pyridinyl substituents in position 2 of thiazole were obtained (**Scheme 1**). For this purpose, the interaction of the corresponding thioureas **1a–c** with 2-chloromalondialdehyde was used. In addition, to study the effect of the halogen on the electronic structure revealed in the absorption and luminescence spectra in position 4, the corresponding 4-bromo- and 4-chloro-5-formylthiazoles **4d–f** were obtained [21, 22]. Finally, to study the impact of a *bis*-heterocyclic system, we synthesized compounds **4g,h** using 2,4-dibromo-5-formylthiazole as a starting compound, according to our previously developed method [23], followed by the removal of the dioxolane protection [24]. Detailed conditions for the synthesis of each of the compounds studied are given below.

At the next stage, by the condensation of formylthiazoles **4** with 2-hydroxyacetophenone (**7**) the corresponding hetarylpropenones **5** were obtained (**Scheme 2**). To prepare the propenones, several methods were tested. The first was the interaction of the starting compounds under the alkaline catalysis (KOH) in methanol and did not lead to the target products. Then we investigated a method using sodium hydride in the DMF medium, and another one with NaOMe in DMF. Both methods made it possible to obtain the desired products with satisfactory yields. Among these two methods, the option with NaOMe was chosen as preferable since it was more convenient as it did not require additional purification of the products from mineral oil impurities.

Then according to the Algar-Flynn-Oyamada method [19, 25], the corresponding hetarylchromones **6** were synthesized. Solutions of potassium



Conditions: ***A****: chlormalondialdehyde, HOAc, NaOAc, 100 °C, 3 hours;* ***B****: R_2NH CH_3CN/H_2O, 10 °C, 30 min;* ***C****: 1) ethylene glycol, TosOH, toluene, 115 °C, 24 hours, 2) $RSnBu_3$, DMF, CuI, Pd G3 AmPhos, 100 °C, 24 hours, 3) oxalic acid, SiO_2, CH_2Cl_2, 20 °C, 48 hours*

Scheme 1. The synthesis of 5-formylthiazoles

ISSN 2308-8303 (Print) / 2518-1548 (Online)

hydroxide and 30% hydrogen peroxide were added to a solution of the corresponding chalcone analog in methanol. At first, a precipitate of epoxy derivatives was formed, and it was dissolved over time. Due to the amphoteric nature of the products, neutralization was carried out with the calculated amount of acetic acid.

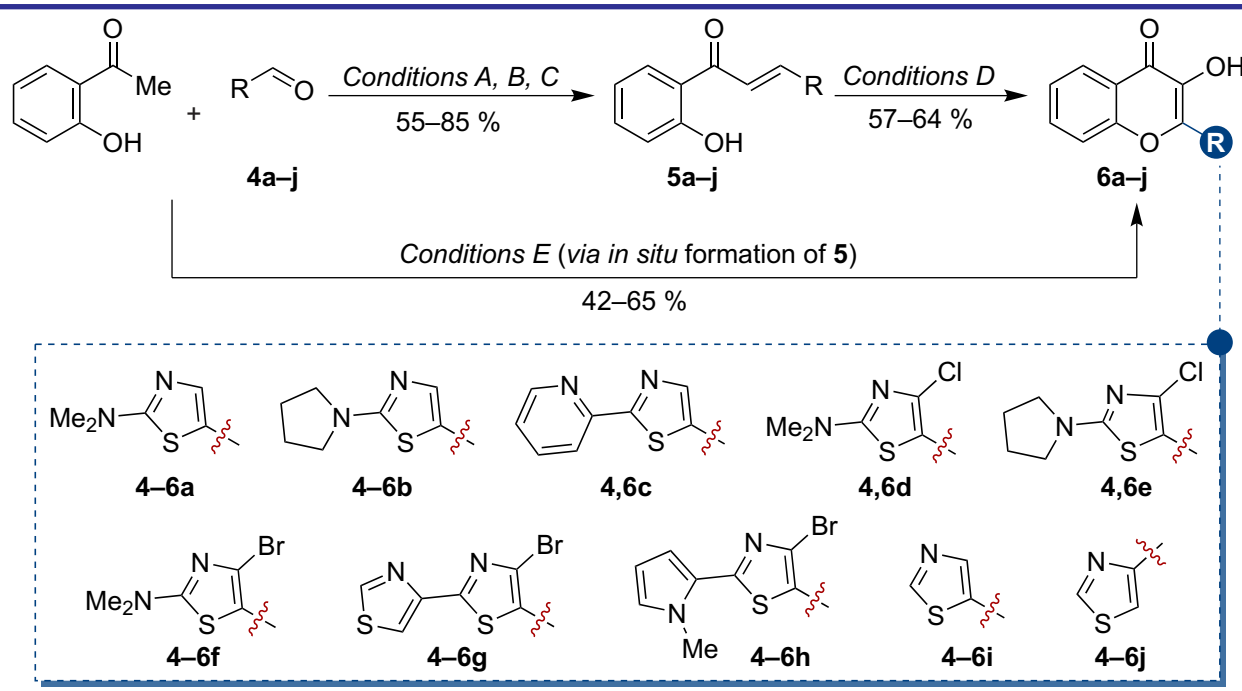
One should mention that for some derivatives the preparation of the intermediate chalcone analogs had some difficulties (compounds **5c–e**). In these cases, upon neutralization with acetic acid, a resinous substance was formed. It was a mixture of the target chalcone analog and other unidentified condensation products. It was not possible to separate the required chalcone from by-products, and their isolation was considered inappropriate. In order to reach our purpose of obtaining compounds **6c–e**, we proposed an alternative synthetic approach. It included the formation of unsaturated ketones *in situ* and the effect of the reaction medium to an oxidizing agent (hydrogen peroxide) in the alkaline medium (**Scheme 2**, *Method E*). Moreover, it was found that the yield of products **6** using the one-pot approach was only slightly lower as compared to the two-stage sequence with the isolation of intermediate chalcones. Thus, the one-pot yield of the final hetarylchromones **6a,f,i** differed from the stepwise one obtained by the stepwise transformation by 4–7%.

Conclusions

The synthetic approach to thiazole-containing chromones with amino, halogen and heterocyclic substituents has been developed. The method can be easily used to extend the series of related heterocyclic chromones. It has been shown that a direct one-pot transformation from thiazole-5-carbaldehyde to chromone is advantageous comparing to a two-step transformation with the isolation of the intermediate chalcone analogs.

Experimental part

All chemicals, unless otherwise stated, were obtained from Enamine Ltd. and used without further purification. Products **4a-f** were synthetized as described in [22, 26], substances **4i,j** were received from commercial sources. All solvents were purified by standard methods. All procedures were carried out at 1 atm. with no precautions taken to exclude ambient moisture. Melting points of all the compounds synthesized were determined with a Gallenkamp melting point apparatus in open capillary tubes. ^{1}H NMR spectra were recorded on a Varian MR-400 spectrometer (400 MHz) with TMS as an internal standard. ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 (126 MHz) spectrometer with TMS as an internal standard. HPLC-MS spectra were recorded



Conditions: ***A****: DMF, NaOMe, 20 °C 2 hours;* ***B****: MeOH, KOH, 20 °C, 2 hours;* ***C****: DMF, NaH, 0 °C 2 hours;* ***D****: 1) MeOH, KOH, 20 °C, 10 min, 2) H_2O_2 (30% aqueous), 20 °C, 3 hours;* ***E****: 1) MeOH, KOH, 10 hours, 2) KOH, H_2O_2 (30% aqueous), 65 °C, 1 hour*

Scheme 2. The synthesis of thiazole-containing chromones

using the chromatography/mass-spectrometric system consisting of a high-performance liquid chromatograph Agilent 1100 LC MSD SL instrument equipped with a diode-matrix and mass-selective detector "Agilent LC/MSD SL". The parameters of the chromatography-mass analysis were the column – SUPELCO Ascentis Express C18, 2.7 μm 4.6 mm×15 cm. According to the HPLC-MS data, all the compounds synthesized had purity > 95%. The elemental analysis was performed in the Institute of Organic Chemistry of the NASU. Absorption spectra were measured on a Hitachi U3210 spectrophotometer, fluorescence spectra were measured on a Hitachi 850 spectrofluorimeter in a concentration of the compounds studied of 10^{-5}–10^{-6} mol/l in acetonitrile and a thickness of the absorbing layer of 1 cm.

The general procedure for the synthesis of compounds 4g,h (Method C)

First two stages (dioxolane protection and the Stille reaction) were performed as described in our previous work [23].

A solution (1 mmol) of the corresponding dioxolane-protected aldehyde in 20 mL of CH_2Cl_2 and 0.25 mL of a 5% aqueous solution of oxalic acid were added to 5 g of SiO_2 and stirred for 24 h. Then the mixture was filtered through a layer of Na_2SO_4, and the solvent was removed on a rotary evaporator.

4-Bromo-[2,4'-bithiazole]-5-carbaldehyde (**4g**)

A yellow solid. Yield – 83%. Anal. Calcd for $C_7H_3BrN_2OS_2$, %: C 30.56, H 1.10, N 10.18, S 23.30. Found, %: C 30.52, H 1.12, N 10.15, S 23.32. ^{1}H NMR (400 MHz, Chloroform-*d*), *δ*, ppm: 8.24 (1H, d, *J* = 2.0 Hz, S-CH=N-C=C<u>H</u>), 8.88 (1H, d, *J* = 2.1 Hz, S-C<u>H</u>=N), 10.01 (1H, s, C<u>H</u>O). Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 274 (100), 276 (98).

4-Bromo-2-(1-methyl-1H-pyrrol-2-yl)thiazole-5-carbaldehyde (**4h**)

A yellow solid. Yield – 87%. Anal. Calcd for $C_8H_6BrN_3OS$, %: C 35.31, H 2.22, N 15.44, S 11.78. Found, %: C 35.33, H 2.24, N 15.42, S 11.77. ^{1}H NMR (400 MHz, Chloroform-*d*), *δ*, ppm: 4.02 (3H, s, C<u>H</u>$_3$), 6.19 (1H, dd, *J* = 4.1, 2.5 Hz, N-CH=C<u>H</u>-CH), 6.83 (1H, s, N-CH=CH-C<u>H</u>), 6.89 (1H, dd, *J* = 4.1, 1.7 Hz, N-C<u>H</u>=CH-CH), 9.91 (1H, s, C<u>H</u>O). ^{13}C NMR (101 MHz, Chloroform-*d*), *δ*, ppm: 37.44, 98.42, 109.86, 116.53, 125.49, 128.67, 130.26, 135.28, 182.61. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 270 (100), 272 (98).

The general procedure for the synthesis of compounds 5a,b,f–i (Conditions C)

2-Hydroxyacetophenone (1 mmol) and the corresponding aldehyde (1 mmol) were dissolved in 15 mL of DMF. Sodium methoxide (3 mmol) was added, and the reaction mixture was stirred for 2 h. Water (30 mL) was added to the solution, and then it was neutralized with acetic acid (3 mmol). The precipitate formed was filtered and washed with water.

(E)-3-(2-(Dimethylamino)thiazol-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5a**)

A yellow solid. Yield – 84%. M. p. 178 °C. Anal. Calcd for $C_{14}H_{14}N_2O_2S$, %: C 61.29, H 5.14, N 10.21, S 11.69. Found, %: C 61.32, H 5.11, N 10.19, S 11.69. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.11 (6H, s, 2C<u>H</u>$_3$), 6.89 (2H, dd, *J* = 7.7, 6.1 Hz, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.09 (1H, d, *J* = 14.7 Hz, C(O)-C<u>H</u>=CH), 7.46 (1H, t, *J* = 7.8 Hz, C(OH)=C<u>H</u>-CH=CH-CH), 7.79 (1H, s, S-C=N-C<u>H</u>=C), 7.92 (1H, d, *J* = 14.7 Hz, C(O)-CH=C<u>H</u>), 8.04 (1H, d, *J* = 8.4 Hz, C(OH)=CH-CH=CH-C<u>H</u>), 12.87 (1H, s, O<u>H</u>). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 39.99, 115.48, 118.12, 119.43, 120.90, 124.39, 130.63, 136.16, 137.40, 151.84, 162.38, 173.32, 192.68. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 274 (100).

(E)-1-(2-Hydroxyphenyl)-3-(2-(pyrrolidin-1-yl)thiazol-5-yl)prop-2-en-1-one (**5b**)

A yellow solid. Yield – 75%. M. p. 210 °C. Anal. Calcd for $C_{16}H_{16}N_2O_2S$, %: C 63.98, H 5.37, N 9.33, S 10.67. Found, %: C 63.94, H 5.33, N 9.35, S 10.70.^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.03 (4H, s, N-CH$_2$-C<u>H</u>$_2$-), 3.50 (4H, s, N-C<u>H</u>$_2$-CH$_2$-), 6.95 (2H, s, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.15 (1H, d, *J* = 14.7 Hz, C(O)-C<u>H</u>=CH), 7.52 (1H, s, C(OH)=C<u>H</u>-CH=CH-CH), 7.87 (1H, s, C(OH)=CH-CH=CH-C<u>H</u>), 8.00 (1H, d, *J* = 14.7 Hz, C(O)-CH=C<u>H</u>), 8.11 (1H, s, S-C=N-C<u>H</u>=C), 12.97 (1H, s, O<u>H</u>). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 25.19, 51.11, 118.18, 120.04, 121.15, 124.17, 129.29, 130.49, 135.38, 136.18, 140.66, 151.96, 162.18, 193.42. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 300 (100).

(E)-3-(4-Bromo-2-(dimethylamino)thiazol-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5f**)

A yellow solid. Yield – 70%. M. p. 190 °C. Anal. Calcd for $C_{14}H_{13}BrN_2O_2S$, %: C 47.60, H 3.71, N 7.93, S 9.08. Found, %: C 46.58, H 3.72, N 7.97, S 9.06. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.15 (6H, s, 2C<u>H</u>$_3$), 6.94 (2H, dd, *J* = 8.5, 7.3 Hz, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.21 (1H, d, *J* = 14.7 Hz, C C(O)-C<u>H</u>=CH), 7.47–7.57 (1H, m, C(OH)=C<u>H</u>-CH=CH-CH), 7.78 (1H, d, *J* = 14.7 Hz, C(O)-CH=C<u>H</u>), 8.05–8.13 (1H, m, C(OH)=CH-CH=CH-C<u>H</u>), 12.68 (1H, s, O<u>H</u>). ^{13}C NMR (151 MHz, DMSO-d_6), δ, ppm: 39.09, 118.18, 120.06, 121.16, 123.86, 126.64, 127.72, 130.32, 134.84, 135.33, 162.51, 167.04,

ISSN 2308-8303 (Print) / 2518-1548 (Online)

192.90. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 351 (100), 353(97).

(E)-3-(4-Bromo-[2,4'-bithiazol]-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5g**)

A yellow solid. Yield – 55%. M. p. 172 °C. Anal. Calcd for $C_{15}H_9BrN_2O_2S_2$, %: C 45.81, H 2.31, N 7.12, S 16.30. Found, %: C 45.79, H 2.33, N 7.10, S 8.17. ^{1}H NMR (500 MHz, Chloroform-*d*), δ, ppm: 6.93 (1H, d, *J* = 15.3 Hz, C(O)-C<u>H</u>=CH), 7.00 (1H, d, *J* = 8.4 Hz, C(OH)=CH-CH=CH-C<u>H</u>), 7.44–7.53 (2H, m, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.81 (1H, d, *J* = 8.1 Hz, C(OH)=C<u>H</u>-CH=CH-CH), 7.99 (1H, d, *J* = 15.3 Hz, C(O)-CH=C<u>H</u>), 8.18 (1H, s, N-CH=CH-C<u>H</u>), 8.87 (1H, s, S-C<u>H</u>=N), 12.65 (1H, s, O<u>H</u>). ^{13}C NMR (126 MHz, Chloroform-*d*), δ, ppm: 118.21, 118.54, 119.19, 122.49, 128.94, 130.47, 132.67, 133.43, 136.25, 148.28, 153.61, 162.08, 163.15, 170.62, 191.80. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 392 (100), 394 (98).

(E)-3-(4-Bromo-2-(1-methyl-1H-pyrrol-2-yl)thiazol-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5h**)

A yellow solid. Yield – 65%. M. p. 195 °C. Anal. Calcd for $C_{17}H_{13}BrN_2O_2S$, %: C 52.45, H 3.37, N 7.20, S 8.24. Found, %: C 52.49, H 3.33, N 7.18, S 8.21. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.79 (3H, s, C<u>H</u>$_3$), 6.04 (1H, dd, *J* = 4.1, 2.6 Hz, N-CH=C<u>H</u>-CH), 6.72 (1H, dd, *J* = 4.1, 1.7 Hz, N-CH=CH-C<u>H</u>), 6.82 (2H, t, *J* = 8.2 Hz, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.00 (1H, d, *J* = 2.2 Hz, N-CH=CH-C<u>H</u>), 7.36–7.41 (1H, m, C(OH)=CH-CH=CH-C<u>H</u>), 7.46 (1H, d, *J* = 15.2 Hz, C(O)-C<u>H</u>=CH), 7.62 (1H, d, *J* = 15.2 Hz, C(O)-CH=C<u>H</u>), 7.90–7.98 (1H, m, C(OH)=C<u>H</u>-CH=CH-CH), 12.04 (1H, s, O<u>H</u>). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 36.68, 109.44, 115.25, 117.66, 119.23, 120.97, 124.15, 124.77, 126.54, 130.42, 130.68, 132.20, 132.60, 136.22, 143.03, 161.67, 191.72. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 388 (100), 390 (98).

(E)-1-(2-Hydroxyphenyl)-3-(thiazol-5-yl)prop-2-en-1-one (**5i**)

A yellow solid. Yield – 81%. M. p. 111 °C. Anal. Calcd for $C_{12}H_9NO_2S$, %: C 62.32, H 3.92, N 6.06, S 13.86. Found, %: C 62.35, H 3.97, N 6.00, S 13.89. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.99 (2H, t, *J* = 7.6 Hz, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.55 (1H, t, *J* = 7.2 Hz, C(OH)=C<u>H</u>-CH=CH-CH), 8.12 (1H, d, *J* = 7.6 Hz, C(OH)=CH-CH=CH-C<u>H</u>), 8.07 (1H, d, *J* = 15.3 Hz, C(O)-C<u>H</u>=CH), 7.74 (1H, d, *J* = 15.3 Hz, C(O)-CH=C<u>H</u>), 8.44 (1H, s, S-CH=N-C<u>H</u>=C), 9.26 (1H, s, S-C<u>H</u>=N), 12.22 (1H, s, OH). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 117.64, 119.21, 120.94, 124.16, 130.76, 133.88, 135.17, 136.20, 148.27, 157.50, 161.33, 192.43. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 232 (100).

(E)-1-(2-Hydroxyphenyl)-3-(thiazol-4-yl)prop-2-en-1-one (**5j**)

A yellow solid. Yield – 85%. M. p. 110-112 °C. Anal. Calcd for $C_{12}H_9NO_2S$, %: C 62.32, H 3.92, N 6.06, S 13.86. Found, %: C 62.29, H 3.95, N 6.03, S 13.89. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.99 (2H, t, *J* = 8.0 Hz, C(OH)=CH-C<u>H</u>=C<u>H</u>-CH), 7.48–7.63 (1H, m, C(OH)=C<u>H</u>-CH=CH-CH), 7.84 (1H, d, *J* = 15.2 Hz, C(O)-C<u>H</u>=CH), 7.98 (1H, d, *J* = 2.8 Hz, C(OH)=CH-CH=CH-C<u>H</u>), 8.01 (1H, d, *J* = 15.2 Hz, C(O)-CH=C<u>H</u>), 8.32 (1H, d, *J* = 1.8 Hz, S-CH=N-C=C<u>H</u>), 9.23 (1H, d, *J* = 1.8 Hz, S-C<u>H</u>=N), 12.16 (1H, s, O<u>H</u>). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 118.21, 119.86, 121.88, 124.02, 125.63, 130.96, 136.56, 136.89, 152.66, 156.31, 161.65, 193.87. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 232 (100).

The general procedure for the synthesis of compounds 6a,b,e,f–i (Conditions D)

The corresponding propenone (1 mmol) was dissolved in methanol, then while stirring potassium hydroxide (3 mmol) was added to the solution. After 10 min of stirring 30% aqueous hydrogen peroxide (3 mmol) was added, and the reaction mixture was stirred for 3 more h. Then mixture was neutralized with acetic acid (3 mmol), and the formed precipitate was filtered and washed with methanol.

The one-pot procedure for the synthesis of compounds 6a,c,d,e,f,i (Conditions E)

2-Hydroxyacetophenone (1 mmol) and the corresponding aldehyde (1 mmol) were dissolved in methanol (10 mL), a catalytic amount of potassium hydroxide was added, and the reaction mixture was stirred for 10 h. Then potassium hydroxide (2 mmol) and 2 mL of a 30% aqueous hydrogen peroxide was added. The resulting mixture was refluxed for 1 h, cooled and neutralized with hydrochloric acid (2 mmol). The precipitate formed was filtered off and washed with methanol and water.

2-(2-(Dimethylamino)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6a**)

A yellow solid. Yield – 60% (48% for the Method H). M. p. 255 °C (decomp.). Anal. Calcd for $C_{14}H_{12}N_2O_3S$, %: C 58.32, H 4.20, N 9.72, S 11.12. Found, %: C 58.30, H 4.24, N 9.76, S 11.10. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.00 (6H, s, 2×C<u>H</u>$_3$), 6.93 (2H, s, C(-O-)=CH-C<u>H</u>=C<u>H</u>-CH), 7.21 (1H, s, C(-O-)=CH-CH=CH-C<u>H</u>), 7.49 (1H, s, S-CH=N-C<u>H</u>=C), 8.07 (1H, s, C(-O-)=C<u>H</u>-CH=CH-CH). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 39.44, 114.75, 117.75, 121.97, 124.35, 124.62, 133.00, 134.67, 143.61, 153.92, 170.71, 173.27. Mass

spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 288 (100). $\lambda_{a\ max}$ (nm) = 400, $\lambda_{f\ max}$ (nm) = 580.

3-Hydroxy-2-(2-(pyrrolidin-1-yl)thiazol-5-yl)-4H-chromen-4-one (**6b**)

A yellow solid. Yield – 63%. M. p. 261 °C decomp. Anal. Calcd for $C_{16}H_{14}N_2O_3S$, %: C 61.13, H 4.49, N 8.91, S 10.20. Found, %: C 61.15, H 4.45, N 8.94, S 10.25. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.01 (4H, s, N-CH_2-C$\underline{H}_2$-), 3.46 (4H, s, N-C$\underline{H}_2$-CH_2-), 7.41 (1H, s, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.61 (1H, s, C(-O-)=CH-CH=CH-C$\underline{H}$), 7.71 (C(-O-)=CH-C$\underline{H}$=CH-CH), 8.01 (1H, s, S-CH=N-C$\underline{H}$=C), 8.05 (1H, s, C(-O-)=C$\underline{H}$-CH=CH-CH). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 25.66, 49.88, 114.66, 118.27, 122.53, 124.86, 125.13, 133.49, 135.06, 143.71, 144.30, 154.44, 170.15, 171.14. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 314 (100). $\lambda_{a\ max}$ (nm) = 405, $\lambda_{f\ max}$ (nm) = 580.

3-Hydroxy-2-(2-(pyridin-2-yl)thiazol-5-yl)-4-chromen-4-one (**6c**) *(Method H)*

A yellow solid. Yield – 46%. M. p. 255 °C (decomp.). Anal. Calcd for $C_{17}H_{10}N_2O_3S$, %: C 63.35, H 3.13, N 8.69, S 9.95. Found, %: C 63.32, H 3.14, N 8.65, S 9.93. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.48 (1H, t, *J* = 7.6 Hz, N-CH=C$\underline{H}$-CH=CH), 7.51–7.59 (1H, m, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.76 (1H, d, *J* = 8.5 Hz, C(-O-)=C$\underline{H}$-CH=CH-CH), 7.82 (1H, t, *J* = 7.8 Hz, C(-O-)=CH-C$\underline{H}$=CH-CH), 8.00 (1H, t, *J* = 7.8 Hz, N-CH=CH-C$\underline{H}$=CH), 8.11 (1H, d, *J* = 8.0 Hz, N-CH=CH-CH=C$\underline{H}$), 8.20 (1H, d, *J* = 7.8 Hz, C(-O-)=CH-CH=CH-C$\underline{H}$), 8.67 (1H, d, *J* = 5.7 Hz, N-C$\underline{H}$=CH-CH=CH), 8.69 (1H, s, CH(Thz)), 10.72 (1H, s, O$\underline{H}$). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 117.55, 120.97, 122.57, 123.00, 124.25, 124.87, 125.62, 125.72, 134.38, 134.89, 137.30, 140.97, 142.55, 145.39, 149.69, 152.65, 162.61, 175.35. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 322 (100). $\lambda_{a\ max}$ (nm) = 380, $\lambda_{f\ max}$ (nm) = 570.

2-(4-Chloro-2-(dimethylamino)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6d**) *(Method H)*

A yellow solid. Yield – 43%. M. p. 254 °C (decomp.). Anal. Calcd for $C_{14}H_{11}ClN_2O_3S$, %: C 52.10, H 3.44, N 8.68, S 9.93. Found, %: C 52.13, H 3.42, N 8.65, S 9.97. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.08 (6H, d, *J* = 6.2 Hz, 2C$\underline{H}_3$), 7.43 (1H, t, *J* = 7.8 Hz, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.60 (1H, d, *J* = 8.5 Hz, C(-O-)=C$\underline{H}$-CH=CH-CH), 7.74 (1H, t, *J* = 6.9 Hz, C(-O-)=CH-C$\underline{H}$=CH-CH), 8.06 (1H, d, *J* = 8.0 Hz, C(-O-)=CH-CH=CH-C$\underline{H}$). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 39.30, 105.34, 117.78, 121.91, 124.53, 124.63, 133.35, 135.91, 137.26, 141.53, 153.91, 169.53, 171.11. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 322 (100), 324(33). $\lambda_{a\ max}$ (nm) = 390, $\lambda_{f\ max}$ (nm) = 580.

2-(4-Chloro-2-(pyrrolidin-1-yl)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6e**) *(Method H)*

A yellow solid. Yield – 65%). M. p. 255 °C (decomp.). Anal. Calcd for $C_{16}H_{13}ClN_2O_3S$, %: C 55.10, H 3.76, N 8.03, S 9.19. Found, %: C 55.11, H 3.80, N 8.01, S 9.24. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.99 (4H, s, N-CH_2-C$\underline{H}_2$-), 3.40 (4H, s, N-C$\underline{H}_2$-CH_2-), 7.38 (1H, s, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.57 (1H, s, C(-O-)=C$\underline{H}$-CH=CH-CH), 7.68 (1H, s, C(-O-)=CH-C$\underline{H}$=CH-CH), 8.04 (1H, s, C(-O-)=CH-CH=CH-C$\underline{H}$). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 25.17, 49.65, 115.08, 117.77, 122.59, 124.33, 124.96, 134.49, 134.86, 136.78, 143.68, 153.85, 162.33, 175.27. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 348 (100), 350(33). $\lambda_{a\ max}$ (nm) = 395, $\lambda_{f\ max}$ (nm) = 585.

2-(4-Bromo-2-(dimethylamino)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6f**)

A yellow solid. Yield – 64% (42% for the Method H). M. p. 259 °C (decomp.). Anal. Calcd for $C_{14}H_{11}BrN_2O_3S$, %: C 45.79, H 3.02, N 7.63, S 8.73. Found, %: C 45.81, H 2.98, N 7.67, S 8.75. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 3.09 (6H, s, 2C$\underline{H}_3$), 7.44 (1H, t, *J* = 7.4 Hz, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.61 (1H, d, *J* = 8.5 Hz, C(-O-)=C$\underline{H}$-CH=CH-CH), 7.75 (1H, t, *J* = 6.9 Hz, C(-O-)=CH-C$\underline{H}$=CH-CH), 8.07 (1H, d, *J* = 8.1 Hz, C(-O-)=CH-CH=CH-C$\underline{H}$), 10.11 (1H, s, OH). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 39.29, 107.62, 117.71, 121.93, 124.57, 124.68, 125.28, 133.42, 136.27, 153.93, 170.65, 171.28. Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 366 (100), 368(97). $\lambda_{a\ max}$ (nm) = 395, $\lambda_{f\ max}$ (nm) = 585.

2-(4-Bromo-2-(thiazol-4-yl)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6g**)

A yellow solid. Yield – 57%. M. p. 265 °C (decomp.). Anal. Calcd for $C_{15}H_7BrN_2O_3S_2$, %: C 44.24, H 1.73, N 6.88, S 15.74. Found, %: C 44.26, H 1.78, N 6.82, S 15.70. ^{1}H NMR (500 MHz, DMSO-d_6), δ, ppm: 6.73–6.57 (2H, m, C(-O-)=C$\underline{H}$-CH=C$\underline{H}$-CH), 7.16 (1H, t, *J* = 7.6 Hz, C(-O-)=CH-C$\underline{H}$=CH-C$\underline{H}$), 7.45 (1H, s, S-CH=N-C=C$\underline{H}$), 7.70 (1H, d, *J* = 7.7 Hz, C(-O-)=CH-CH=CH-C$\underline{H}$), 9.25 (1H, s, S-C$\underline{H}$=N). Mass spectrum, *m/z* (I_{rel}, %): $[M+H]^+$ 407 (100), 409(98).

2-(4-Bromo-2-(1-methyl-1H-pyrrol-2-yl)thiazol-5-yl)-3-hydroxy-4H-chromen-4-one (**6h**)

A yellow solid. Yield – 60%. M. p. 265 °C (decomp.). Anal. Calcd for $C_{17}H_{11}BrN_2O_3S$, %: C 50.64, H 2.75, N 6.95, S 7.95. Found, %: C 50.68, H 2.79, N 6.90, S 7.97. ^{1}H NMR (400 MHz, Chloroform-*d*), δ, ppm: 4.04 (3H, s, CH_3), 6.18 (1H, s, N-CH=C$\underline{H}$-CH), 6.80 (2H, d, *J* = 3.3 Hz, N-C$\underline{H}$=CH-C$\underline{H}$), 7.22–7.38 (1H, m, C(-O-)=CH-CH=C$\underline{H}$-CH), 7.62 (1H, d, *J* = 8.8 Hz, C(-O-)=C$\underline{H}$-CH=CH-CH), 7.71 (1H, d,

ISSN 2308-8303 (Print) / 2518-1548 (Online)

J = 7.7 Hz, C(-O-)=CH-CH=CH-CH), 8.23 (1H, d, J = 7.9 Hz, C(-O-)=CH-CH=CH-CH). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 34.22, 108.02, 112.15, 117.71, 121.98, 122.62, 124.36, 124.88, 125.67, 126.03, 134.46, 134.86, 142.86, 153.29, 153.48, 175.10. Mass spectrum, m/z (I_{rel}, %): $[M+H]^+$ 402 (100), 404(98). $\lambda_{a\,max}$ (nm) = 400, $\lambda_{f\,max}$ (nm) = 600.

3-Hydroxy-2-(thiazol-5-yl)-4H-chromen-4-one (**6i**)

A white solid. Yield – 64% (49% for the Method H). M. p. 230 °C (decomp.). Anal. Calcd for $C_{12}H_7NO_3S$, %: C 58.77, H 2.88, N 5.71, S 13.07. Found, %: C 58.73, H 2.91 N 5.74, S 13.09. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.47 (1H, t, J = 7.5 Hz, C(-O-)=CH-CH=CH-CH), 7.74 (1H, d, J = 8.6 Hz, C(-O-)=CH-CH=CH-CH), 7.81 (1H, t, J = 7.7 Hz, C(-O-)=CH-CH=CH-CH), 8.11 (1H, d, J = 8.0 Hz, C(-O-)=CH-CH=CH-CH), 8.67 (1H, s, S-CH=N-CH=C),9.33 (1H, s, S-CH=N). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 118.20, 121.79, 124.66, 124.84, 127.77, 133.85, 137.46, 141.45, 143.20, 154.25, 157.63, 172.08. Mass spectrum, m/z (I_{rel}, %): $[M+H]^+$ 245 (100). $\lambda_{a\,max}$ (nm) = 350, $\lambda_{f\,max}$ (nm) = 535.

3-Hydroxy-2-thiazol-4-yl-chromen-4-one (**6j**)

A white solid. Yield (62%), mp. 229-232 °C Anal. Calcd for $C_{12}H_7NO_3S$, %: C 58.77, H 2.88, N 5.71, S 13.07. Found, %: C 58.74, H 2.91, N 5.75, S 13.09. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.48 (t, J = 7.3 Hz, 1H, C(-O-)=CH-CH=CH-CH), 7.72 (dd, J = 8.5, 4.2 Hz, 1H, C(-O-)=CH-CH=CH-CH), 7.77–7.88 (m, 1H, C(-O-)=CH-CH=CH-CH), 8.13 (dd, J = 8.4, 4.2 Hz, 1H, C(-O-)=CH-CH=CH-CH), 8.56 (dd, J = 4.3, 2.0 Hz, 1H, S-CH=N-C=CH), 9.35 (dd, J = 4.3, 2.0 Hz, 1H, S-CH=N). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 118.31, 121.88, 122.95, 124.51, 124.85, 133.75, 138.99, 141.54, 146.29, 154.27, 155.01, 172.45. Mass spectrum, m/z (I_{rel}, %): $[M+H]^+$ 246 (100). $\lambda_{a\,max}$ (nm) = 340, $\lambda_{f\,max}$ (nm) = 515.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)

Journal of Organic and Pharmaceutical Chemistry

Original Research

OPEN ACCESS
Freely available online on
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UDC 547.831

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A Simple Preparative Synthesis of Isomeric 2-Chloroquinolinecarboxylic Esters

Abstract

A simple two-stage method for the synthesis of isomeric esters of 2-chloroquinoline-5-, 6-, 7-carboxylic acids by successive oxidation and chlorination reactions of methyl quinoline-5-, 6-, 7-carboxylates has been developed. The target compounds have been obtained in acceptable yields using readily available reagents, simple transformations, and purification methods. Quinoline-8-carboxylic acid ester is unreactive under these conditions. The ester of 2-chloroquinoline-8-carboxylic acid has been obtained with an overall yield of 55 %, starting from 8-methylquinoline. The multi-stage process is paid off by the fact that several transformations occur in one reaction cycle. All the methods developed can be used for the synthesis of target compounds on a multigram scale. Intermediate 2(1*H*)-oxoquinoline carboxylates are promising compounds in the synthesis of functionalized and condensed heterocycles.

Keywords: 2-chloroquinoline; esters; oxidation; quinolone-2; chlorination

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Простий препаративний синтез ізомерних 2-хлорхінолінкарбонових естерів

Анотація

Розроблено простий двостадійний метод синтезу ізомерних естерів 2-хлорохінолін-5-, 6-, 7-карбонових кислот за допомогою послідовних реакцій окиснення і хлорування метилхінолін-5-, 6-, 7-карбоксилатів. Цільові сполуки було одержано з прийнятними виходами з використанням доступних реагентів, простих перетворень і методів очищення. Естер хінолін-8-карбонової кислоти в цих умовах є нереакційноздатним. Естер 2-хлорхінолін-8-карбонової кислоти було одержано із 8-метилхіноліну з загальним виходом 55 %. Багатостадійність процесу окупається тим, що в одному реакційному циклі відбувається кілька перетворень. Усі розроблені методи можна використовувати для синтезу цільових сполук у мультиграмовому масштабі. Проміжні 2(1*H*)-оксохінолінкарбоксилати є перспективними сполуками в синтезі функціоналізованих та конденсованих гетероциклів.

Ключові слова: 2-хлорхінолін; естер; окиснення; хінолон-2; хлорування

Citation: Ishkov, Yu. V.; Veduta, V. V.; Fed'ko, N. F.; Bogdan, N. M. A Simple Preparative Synthesis of Isomeric 2-Chloroquinolinecarboxylic Esters. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 11–17.
https://doi.org/10.24959/ophcj.23.298574
Received: *2 September 2023*; **Revised:** *18 November 2023*; **Accepted:** *25 November 2023*

Funding: The authors received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

Introduction

2-Chloroquinolines with a carboxyl/ester substituted ring are attractive compounds with a great potential for transformation of the quinoline core [1]. A chlorine atom in position 2 can be easily substituted by N-, O- and S-nucleophiles [2], and this ability is widely used in organic and pharmaceutical chemistry. 2-Chloroquinoline derivatives with carboxyl substituents have a wide range of activities, including antimicrobial [3], anti-inflammatory [4], antitumor [5] and antiparasitic ones [6, 7]. There are several methods of introducing halogen into position 2 of the quinoline molecule, but most of them relate to 2-chloroquinoline derivatives with a carboxyl/ester group in the pyridine nucleus [8, 9].

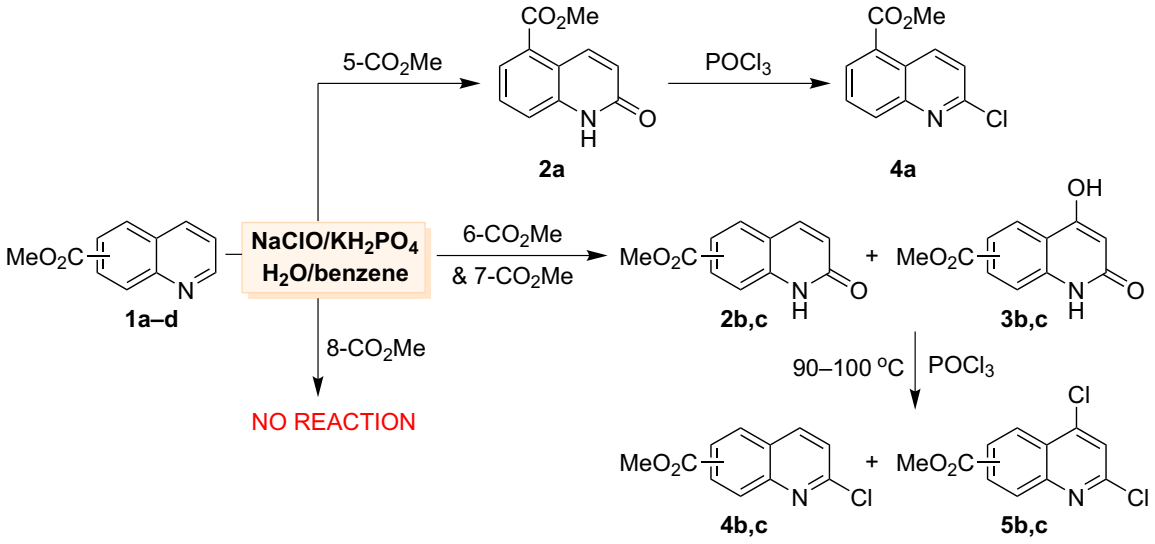
Modern synthetic approaches to 2-chloroquinolines with an ester function in the benzene ring use metal complex catalysts based on ruthenium or iridium [11, 12], which are not always cost effective. Unfortunately, the reaction of C-2 chlorination of N-oxides for quinoline esters in our hands did not give satisfactory results [13, 14]. The aim of this work is to develop simple preparative methods for the 2-chloroquinolines synthesis with ester substituents in positions 5, 6, 7, and 8 of quinoline. Amazingly, all 2-chloroquinolinecarboxylic acids have been known for a long time, but their physical and spectral properties are given in fragments. And we provide known data when it is available.

Results and discussion

We studied the possibilities of optimization for the known classical reactions. The easiest way to obtain the desired compounds is the oxidation of the quinoline 2-position to quinolone-2 followed by the replacement of an oxygen atom with chlorine according to the Friedlander method [15]. Therefore, it seemed attractive to optimize the oxidation reaction of quinolines into quinolone-2 with sodium hypochlorite, an effective low-cost reagent [16]. A series of experiments on the oxidation of 5-, 6-, 7-, and 8-quinoline carboxylate esters **1a–d** with sodium hypochlorite in a two-phase benzene-water system was performed.

In an alkaline solution of hypochlorite (pH ≈ 11), the oxidation of esters **1a–d** did not proceed; therefore, the pH of the solution was adjusted by adding an acidifier potassium dihydrogen phosphate (**Scheme 1**). We obtained the best results with such ratio of $NaClO/KH_2PO_4$ reagents as 2:1.07, while the initial solution had a pH value of 11. After the addition of potassium dihydrogen phosphate and esters **1**, the solution had a pH of 7.5, at the end of the reaction the pH was 5. A fivefold hypochlorite excess was used for the oxidation. The reaction was monitored by the presence of sodium hypochlorite in the reaction mixture. The reaction was not carried out for complete ether **1** conversion since during a prolonged contact the excess hypochlorite also oxidized the target reaction products **2**. The conversion of starting esters **1a–c** reached 80%, and methyl quinoline-8-carboxylate **1d** was unreactive under the reaction conditions.

For better yields of products **2a–c**, sodium thiosulfate $Na_2S_2O_3$ was added to the reaction mixture immediately after exhausting the oxidizing agent. Quinolones-2 **2a,b** precipitated from the reaction mixture, then they were filtered, and impurities were removed by the extraction with



Scheme 1. The synthesis of methyl 2-chloroquinoline carboxylates **4** and 2,4-dichloroquinoline carboxylates **5**

ISSN 2308-8303 (Print) / 2518-1548 (Online)

boiling ethanol. In contrast to compound **1a**, upon the oxidation of esters **1b,c** together with quinolones **2b,c**, minor products were formed – 2,4-dihydroxy derivatives **3b,c**, which were also poorly soluble.

Quinolones **2a–c** under short-term heating with $POCl_3$ [15] were transformed into 2-chloroquinoline esters **4a–c**, in the case of esters **4b,c** – with an impurity of 2,4-dichloro derivatives **5b**,**c**. Monochlorinated **4b** and dechlorinated **5b** quinolines were separated by column chromatography and recrystallized from benzene.

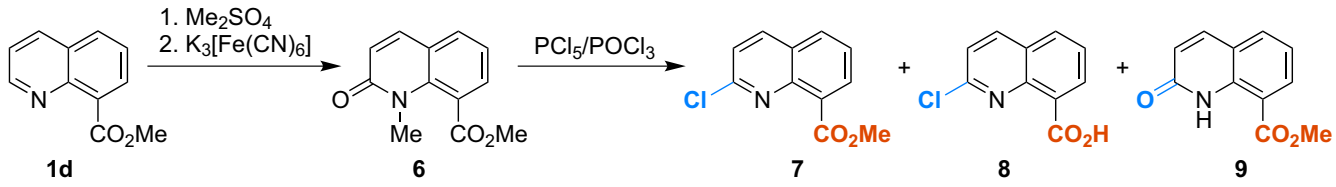
Methyl ester of quinoline-8-carboxylic acid (**1d**) was inert to the $NaClO/KH_2PO_4$ oxidizing system. According to the Dekker's method [17], it was converted into methyl 1-methyl-2-oxoquinoline-8-carboxylate (**6**) with a 42% yield by heating with dimethyl sulfate $(CH_3O)_2SO_2$ at 185 °C for 2.5 h and further oxidation of the quaternary salt with an alkaline solution of potassium hexacyanoferrate(III) at 60 °C (**Scheme 2**). Compound **6** was then converted to methyl 2-chloroquinoline carboxylate **7** according to the Fischer approach [18] by heating it in a mixture of $POCl_3/PCl_5$ at 140 °C with a 40% yield. 2-Chloroquinoline-8-carboxylic acid (**8**) (6%) and 2-oxoquinoline-8-carboxylic acid methyl ester (**9**) (21%) were also isolated along with product **7**.

The low yield of ester **7** and the presence of minor products forced us to develop an alternative route based on 8-methylquinoline (**10**). The alkylation of **10** with dimethyl sulfate and the subsequent oxidation by potassium hexacyanoferrate(III) yielded 1,8-dimethylquinolone-2 (**11**) in one reaction cycle (**Scheme 3**).

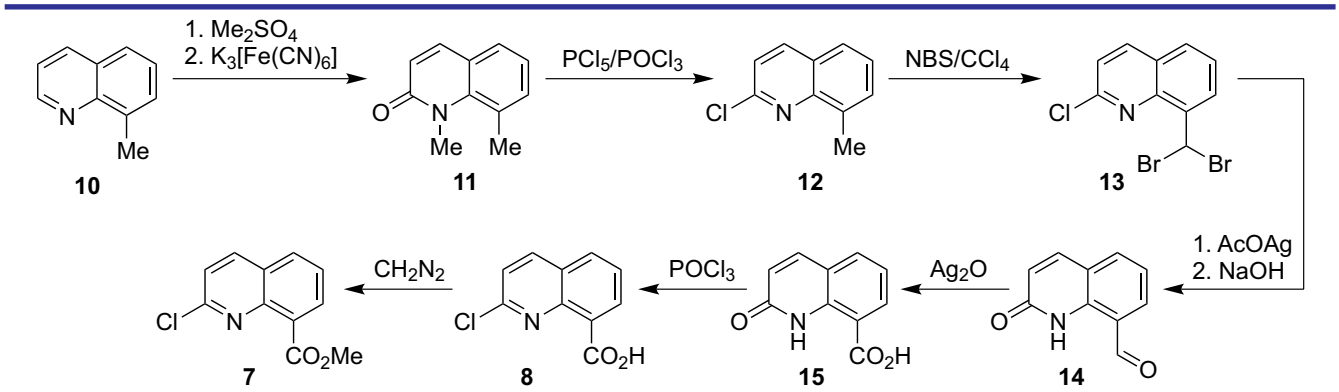
The chlorination of quinolone **11** with a mixture of $PCl_5/POCl_3$ gave 8-methyl-2-chloroquinoline (**12**) [18], which was converted into the corresponding dibromomethyl derivative **13** with N-bromosuccinimide in CCl_4. The hydrolysis of the dibromomethyl group with silver acetate into aldehyde **14** and its oxidation with silver oxide yielded 2-oxoquinoline-8-carboxylic acid (**15**). After the chlorination of acid **15** in $POCl_3$ and the esterification of 2-chloro-8-carboxyquinoline (**8**) with diazomethane in ether, the target ester **7** was obtained. Despite the multistep process, the overall yield of the target product was 55% based on 8-methylquinoline.

■ Conclusions

Simple methods for the synthesis of isomeric esters of 2-chloroquinoline-5-, 6- and 7-carboxylic acids have been developed with acceptable yields using available reagents, simple transformations, and methods of purifying target compounds. A convenient *r*oute for the synthesis of methyl 2-chloroquinoline-8-carboxylate with the total yield of 55% starting from 8-methylquinoline has been developed. The multi-stage process for obtaining this compound is paid off by the fact that several transformations occurred as a telescopic process. All the methods developed can be used for the synthesis of target compounds on a multigram scale. In addition, this



Scheme 2. The synthesis of methyl 2-chloroquinoline-8-carboxylate 7 from methyl quinoline-8-carboxylate



Scheme 3. An optimized reaction set for the methyl 2-chloroquinoline-8-carboxylate synthesis

reaction set provides easy access to useful intermediates with a carboxyl group in a functionalized quinolone core.

■ Experimental part

Control over the course of the reactions, purity and identity of the products obtained was carried out by thin-layer chromatography on Merck 60 F254 plates. ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 solution on a Bruker 170 Avance 500 spectrometer (400 MHz on protons and 100 MHz on carbon atoms, respectively), the internal standard was TMS. Chemical shifts were reported in δ (ppm). Data were presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constants (Hz) and integration. The elemental analysis data corresponded to the calculated data. The melting points were determined on a Fisher-Johns apparatus. A commercial aqueous solution of sodium hypochlorite in 0.305 mol L^{-1} concentration was used. The consumption of the oxidizer was monitored using iodine-starch paper. Melting points of known substances were given if they were found in the literature. Intermediates and non-target substances **5c**, **6**, **9**, **13**, **15** were characterized only by melting points and ^{1}H NMR spectroscopy. The elemental analysis was performed in the Analytical Laboratory of the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine.

Methyl 2-oxoquinoline-5-carboxylate (2a)

Potassium dihydrogen phosphate (15.2 g, 0.112 mol) under vigorous stirring was added to a mixture of the solution of methyl quinoline-5-carboxylate (**1a**) (10 g, 0.053 mol) in 150 mL of benzene and 850 mL (0.255 mol) of the sodium hypochlorite solution at room temperature, and the resulting mixture was stirred for 5 h. Then sodium thiosulfate (53 g, 0.212 mol) was added and stirred for another 3 h. The resulting precipitate was filtered, washed with benzene, water and air-dried. From the benzene mother liquor, 4.8 g of the starting ester **1a** was recovered.

A white powder. Yield – 4.5 g (42%). M. p. ~290 °C. Anal. Calcd for $C_{11}H_9NO_3$, %: C 65.0, H 4.46, N 6.89. Found, %: C 65.1, H 4.40, N 6.94. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.90 (3H, s, MeO), 6.60 (1H, d, J = 10.0 Hz), 7.52 (1H, d, J = 7.2 Hz), 7.63 (1H, t, J = 7.2 Hz), 7.75 (1H, d, J = 7.2 Hz), 8.60 (1H, d, J = 10.0 Hz), 12.0 (1H, br. s, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 52.0 (MeO), 120.0, 124.1, 124.5, 129.5, 137.0, 117.0, 127.8, 140.1, 161.4 (C=O), 167.7 (C=O).

Methyl 2-chloroquinoline-5-carboxylate (4a)

Quinolone **2a** (6.4 g, 0.0315 mol) and 12 mL (0.128 mol) of phosphoryl chloride were heated on a water bath (100 °C) for 20 min. The reaction mass was cooled to room temperature, 25 mL of acetic acid was added, and the mixture was poured onto 500 g of ice. The mixture was made alkaline with NaOH solution (36 g, 0.8 mol) in 75 mL of water, the precipitate was filtered off, washed with water, and dried. The solution of the reaction product in 100 mL of benzene was passed through a layer of Al_2O_3 (5 cm), evaporated to 15 ml, and ~60 mL of heptane was then added. The precipitated crystals were filtered yielding methyl 2-chloroquinoline-5-carboxylate (**4a**).

A yellowish solid. Yield – 5.94 g (87%). M. p. 122–124 °C. Anal. Calcd for $C_{11}H_8ClNO_2$, %: C 59.6, H 3.64, Cl 16.0, N 6.32. Found, %: C 60.0, H 3.55, Cl 16.10, N 6.30. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.93 (3H, s, CH_3), 7.73 (1H, d, J = 9 Hz, 8-H), 7.89 (1H, t, J = 6 Hz, 7-H), 8.18 (1H, d, J = 6 Hz, 6-H), 8.25 (1H, d, J= 6 Hz, 3-H), 9.18 (1H, d, J = 9 Hz, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 52.9, 124.2, 125.3, 127.2, 130.3, 131.2, 133.6, 137.9, 147.8, 150.7, 166.4.

Methyl 2-chloroquinoline-6-carboxylate (4b) and methyl 2,4-dichloroquinoline-6-carboxylate (5b)

The mixture was obtained according to the method described for compound **4a**. 6.6 g of a mixture of quinolones **2b** and **3b** was obtained from 9.5 g (0.051 mol) of ester **1b**, and 1.4 g of the original ester **1b** was recovered. After the reaction of mixture **2b** and **3b** with $POCl_3$, 5.33 g of mixture **4b** and **5b** was obtained, and the components were separated in a column (45×2.5 cm) with silica gel, benzene as an eluent. Methyl 2,4-dichloro-6-carboxylate (**5b**) was eluted first and recrystallized from a mixture of benzene/heptane (1:3). Next, methyl 2-chloro-6-carboxylate (**4b**) was eluted and recrystallized from a mixture of benzene/heptane (1:3).

Methyl 2-chloroquinoline-6-carboxylate (**4b**)

White crystals. Yield – 4.06 g (45%). M. p. 134 °C (lit. M. p. 134–136 °C [13]), R_f = 0.52 (benzene). Anal. Calcd for $C_{11}H_8ClNO_2$, %: C 59.60, H 3.64, Cl 16.00, N 6.32. Found, %: 59.93, H 3.61, Cl 16.00, N 6.28. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.92 (3H, s, OMe), 7.70 (1H, d, J = 9 Hz), 8.03 (1H, d, J = 9 Hz), 8.25 (1H, d, J = 9 Hz), 8.66 (1H, d, J = 9 Hz), 8.74 (1H, s). ^{13}C NMR

ISSN 2308-8303 (Print) / 2518-1548 (Online)

(100 MHz, DMSO-d_6), δ, ppm: 52.9 (MeO), 123.8, 126.5, 128.3, 128.9, 130.2, 130.7, 131.2, 141.7, 149.37, 152.0, 152.7, 165.9 (C=O).

Methyl 2,4-dichloroquinoline-6-carboxylate (**5b**)

Yellowish crystals. Yield – 1.1 g (10%). M. p. 132 °C, R_f = 0.72 (benzene). Anal. Calcd for $C_{11}H_7Cl_2NO_2$, %: C 51.59, H 2.76, Cl 27.69, N 5.47. Found, %: C 51.72, H 2.65, Cl 27.80, N 5.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.91 (3H, s, OMe), 7.99 (1H, d, J = 9 Hz), 8.19 (1H, d, J = 9 Hz), 8.61 (1H, s), 8.91 (1H, s). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 52.9 (MeO), 123.8, 126.5, 128.3, 128.9, 130.2, 130.7, 131.2, 141.7, 149.3, 152.0, 152.7, 165.9 (C=O).

Methyl 2-chloroquinoline-7-carboxylate (4c) and methyl 2,4-dichloroquinoline-7-carboxylate (5c)

The mixture was synthesized according to the method for compound **4b** from 9.0 g (0.048 mol) of ester **1c**. 5.2 g of a mixture of substances **4c** and **5c** were obtained. 2.1 g of the starting ester was isolated from the benzene mother liquor. 5.15 g of a mixture of **4c** and **5c** was dissolved in 400 mL of hot benzene, filtered through a silica gel layer (5 cm) and evaporated to 30 mL. 2,4-Dichloro-7-carboxylate (**5c**), which precipitated after cooling, was filtered off. The mother solution was evaporated to 10 mL, 20 mL of heptane was added, and the precipitate was filtered off giving **4c**.

Methyl 2-chloroquinoline-7-carboxylate (**4c**)

Yellowish crystals. Yield – 4.18 g (49%). M. p. 112–113 °C, R_f = 0.51 ($CHCl_3$). Anal. Calcd for $C_{11}H_8ClNO_2$, %: C 59.6, H 3.64, Cl 16.00, N 6.32. Found, %: C 59.95, H 3.60, Cl 16.00, N 6.30. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.94 (3H, s, MeO), 7.35 (1H, d, J = 10 Hz), 7.70 (1H, d, J = 10 Hz), 8.02-8.07 (2H, m), 8.54 (1H, d, J = 5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 52.7 (MeO), 123.5, 126.6, 128.0, 129.9, 130.2, 130.8, 140.0, 149.2, 153.5, 166.6 (C=O).

Methyl 2,4-dichloroquinoline-7-carboxylate (**5c**)

A yellowish powder. Yield – 0.65 g (6.4%). M. p. 118–120 °C, R_f = 0.67 ($CHCl_3$). Anal. Calcd for $C_{11}H_7Cl_2NO_2$, %: C 51.59, H 2.76, Cl 27.69, N 5.47. Found, %: C 51.65, H 2.68, Cl 27.72, N 5.36. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.91 (3H, s, MeO), 8.11 (1H, s), 8.56 (1H, d, J = 9 Hz), 8.59 (1H, s), 8.78 (1H, d, J = 9 Hz).

Methyl 2-chloroquinoline-8-carboxylate (7). Method 1

Step 1. *Methyl 1-methyl-2(1H)-oxoquinoline-8-carboxylate* (**6**)

Ester **1d** (15.15 g, 0.081 mol) and 26 mL (0.278 mol) of freshly distilled dimethyl sulfate were stirred at 180–185 °C for 2.5 h. After cooling, 50 mL of water was added to the reaction mixture and then poured into the solution of 75 g (0.2278 mol) of potassium hexacyanoferrate(III) in 220 mL of water at 60 °C. Then the solution of NaOH (32 g, 0.8 mol) in 64 mL of water was added to the mixture while stirring for 2-3 min. After cooling, it was extracted with benzene (2×200 mL). The benzene extract was evaporated to 20 mL, and 60 mL of heptane was added. The precipitate was filtered, dried, and compound **6** was thus obtained.

White crystals. Yield – 7.38 g (42%). M. p. 95–96 °C. Anal. Calcd for $C_{12}H_{11}NO_3$, %: C 66.35, H 5.10, N 6.45. Found, %: C 66.40, H 5.05, N 6.40. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.36 (3H, s, N-Me), 3.89 (3H, s, MeO), 6.68 (1H, d, J = 9 Hz), 7.31 (1H, t, J = 9 Hz), 7.75 (1H, d, J = 9 Hz), 7.88 (1H, d, J = 9 Hz), 7.97 (1H, d, J = 12 Hz).

Step 2. *Methyl 2-chloroquinoline-8-carboxylate* (**7**), *2-chloroquinoline-8-carboxylic acid* (**8**), *methyl 2(1H)-2-oxoquinoline-8- carboxylate* (**9**)

Ester **6** (9.7 g, 0.0447 mol) was mixed with phosphorus pentachloride (12.6 g, 0.0605 mol) and phosphorus oxychloride (4.2 mL, 0.0447 mol), and stirred at 140 °C for 1 h. Water and ice were added to the reaction mass, the mixture was neutralized with 20% NaOH to pH 5–6 and extracted with chloroform 3×150 mL. The extract was passed through the aluminum oxide layer (6–7 cm) and evaporated. The residue was dissolved in benzene (~20–25 mL) and introduced into a column with silica gel (2.5×50 cm). Ester **7** was obtained with benzene as an eluent. By changing the eluent to benzene/acetone 50:1, 2-chloroquinoline-8-carboxylate (**8**) and methyl 2(*1H*)-oxoquinoline-8-carboxylate (**9**) were isolated.

Methyl 2-chloroquinoline-8-carboxylate (**7**)

A yellowish oil. Yield – 4 g (40.4%). Anal. Calcd for $C_{11}H_8ClNO_2$, %: C 59.60, H 3.64, Cl 16.00, N 6.32. Found, %: C 59.90, H 3.60, Cl 16.10, N 6.33. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.90 (3H, s, MeO), 7.69 (1H, d, J = 8 Hz), 7.73 (1H, t, J = 8 Hz), 8.05 (1H, d, J = 8 Hz), 8.22 (1H, d, J = 8), 8.54 (1H, d, J = 8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 53.3, 121.4, 121.8, 122.0, 130.2, 132.3, 138.5, 140.1, 162.5, 169.0.

2-Chloroquinoline-8-carboxylic acid (**8**)

A yellowish solid. Yield – 0.55 g (5.9%). M. p. 212–215 °C. Anal. Calcd for $C_{10}H_6ClNO_2$, %: C 57.85, H 2.91, Cl 17.08, N 6.75. Found, %: C 57.90, H 2.87, Cl 17.10, N 6.65. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 7.52–7.76 (2H, m), 8.20–8.28 (2H, m), 8.62 (1H, d, J = 9 Hz), 13.82 (1H, s, COOH).

^{13}C NMR (100 MHz, DMSO-d_6), δ, ppm: 123.55, 127.16, 127.34, 127.48, 132.26, 132.43, 132.78, 133.08, 141.69, 150.55.

Methyl 2(1H)-2-oxoquinoline-8-carboxylate (**9**)

A white solid. Yield – 1.92 g (21%). M. p. 134–136 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.91 (3H, s, MeO), 6.61 (1H, d, J = 9 Hz), 7.28 (1H, t, J = 9 Hz), 7.99-8.04 (2H, m), 8.15 (1H, d, J = 9 Hz), 11.38 (1H, br. s, NH).

Methyl 2-chloroquinoline-8-carboxylate (7). Method 2

Step 1. *2-Chloro-8-dibromomethylquinoline* (**13**)

A mixture of 2-chloro-8-methylquinoline (**12**) (15 g, 0.0845 mol) [16], N-bromosuccinimide (32 g, 0.1798 mol), 150 mL of carbon tetrachloride, and 0.25 g of azo-*bis*-isobutyronitrile was refluxed for 2 h. The reaction was diluted with 150 mL of boiling CCl_4 and quickly filtered, the filter was washed with 50 mL of carbon tetrachloride. The solvent was evaporated to the volume of 120 mL, 150 mL of hot ethanol was added and evaporated to the volume of 110-120 mL. After 12 h, compound **13** was filtered, washed with ethanol (~60 mL) and dried.

A yellow powder. Yield – 21.8 g (76.9%). M. p. 165–170 °C. Dibromo derivative **13** was used without further purification.

Step 2. *2(1H)-Oxoquinoline-8-carboxylic acid* (**15**)

A mixture of 21.8 g (0.065 mol) of dibromide **13** and 53.93 g (0.325 mol) of silver acetate in 150 ml of ethanol was heated to 55 °C and, with stirring, 50 mL of hot (50 °C) water was added. Then the solution of 18.2 g (0.455 mol) of NaOH in 50 mL of water was added dropwise over 20 min. Next, 50 mL portion of hot water was added and stirred for 10 min. The reaction mixture was heated to 60–65 °C, and the stirring was continued for another 40 min, and the heating was removed. After 30 min, the solution was filtered from silver compounds, and the residue on the filter was washed with 200 mL of hot water (60–65 °C). The solution obtained was extracted with benzene (2×200 mL) and neutralized with the solution of 35 mL (0.132 mol) of 10% hydrochloric acid. The precipitate of acid **15** was filtered off and dried.

A white powder. Yield – 9.46 g (77%). M. p. 139–140 °C (lit. M. p. 140–142 °C [19]). ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 6.62 (1H, d, J = 10 Hz), 7.31 (1H, d, J = 7.8 Hz), 7.99 (1H, d, J = 7.8 Hz), 8.04 (1H, d, J = 10 Hz), 8.20 (1H, d, J = 7.8 Hz), 11.8 (1H, br. s, NH).

Step 3. *2-Chloroquinoline-8-carboxylic acid* (**8**)

A mixture of oxoacid **15** (14.7 g, 0.078 mol) and phosphorus oxychloride (17 mL, 0.182 mol) was heated for 20 min under reflux. Then the mixture was cooled to 40–45 °C, 80 mL of glacial acetic acid was added and poured onto crushed ice (~200 g), mineral acids were neutralized with a dry sodium carbonate to pH 5. The precipitate was filtered off, washed with water, dried in air giving 15.2 g (93%) of acid **8**. M. p. 216–219 °C. ^{1}H and ^{13}C NMR spectra were consistent with compound **8** obtained by the **Method 1** and the literature data [20].

Step 4. *Methyl 2-chloroquinoline-8-carboxylate* (**7**)

Acid **8** (14.7 g, 0.0705 mol) was heated to boiling in 650 mL of chloroform and 50 mL of methanol and cooled to 25 °C. The solution of diazomethane (5.6 g, 0.133 mol) in 200 mL of a mixture of benzene/diethyl ether (1:1) was poured into the solution for 10 min. After the release of nitrogen, the excess of diazomethane was decomposed by glacial acetic acid. The resulting solution was washed with water, 300 mL of 3% sodium bicarbonate solution was passed through the aluminum oxide layer (10 cm), the solvent was evaporated in vacuum.

Yield of ester **7** – 15 g (95.6%) as a yellow oil. ^{1}H and ^{13}C NMR spectra were consistent with those for compound **7** obtained by **Method 1** and the literature data [20].

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Journal of Organic and Pharmaceutical Chemistry

Original Research

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UDC 54.057:547.85:615.28

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An Efficient Synthesis of PARP Inhibitors Containing a 4-Trifluoromethyl Substituted 3,6,7,7a-Tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione Scaffold

Abstract

Poly(ADP-ribose) polymerases (PARPs) are key enzymes in the DNA repair pathway. Inhibitors of these enzymes belong to a new type of anticancer drugs that selectively kill cancer cells by targeting the homologous recombination genetic defects. This study presents a new synthetic approach to PARP inhibitors containing a 4-trifluoromethyl substituted 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione scaffold. The method is based on a practical one-step cyclocondensation of 2-(2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid derivatives *via* the Curtius rearrangement of the corresponding acyl azides formed *in situ* upon the treatment with diphenylphosphoryl azide. The resulting products have been found to possess a potent inhibitory effect on PARP-1 and PARP-2 isoforms of poly(ADP-ribose) polymerases. The structure–activity analysis has revealed that the N1-aryl substituent is crucial to the selectivity and high potency towards PARP-2, and that the *p*-fluorobenzyl group is the optimal group for the non-selective and potent PARP-1 and PARP-2 inhibition.

Keywords: Curtius rearrangement; heterocyclization; trifluoromethyl group; pyrrolo[3,4-*d*]pyrimidines; poly(ADP-ribose) polymerase inhibitors

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Ефективний синтез інгібіторів PARP на основі 4-трифторометилзаміщеного скафолду 3,6,7,7a-тетрагідро-1*H*-піроло[3,4-*d*]піримідин-2,5-діону

Анотація

Полі(АДФ-рибоза)-полімерази (PARP) є ключовими ензимами в процесах репарації ДНК. Інгібітори цих ензимів належать до нового типу протипухлинних препаратів, які вибірково вражають ракові клітини, націлюючись на генетичні дефекти гомологічної рекомбінації. У роботі наведено новий синтетичний підхід до інгібіторів PARP, що містять 4-трифторометилзаміщений скафолд 3,6,7,7a-тетрагідро-1*H*-піроло[3,4-d]піримідин-2,5-діону. Метод базується на практичній одностадійній циклоконденсації похідних 2-(2-оксо-1,2,3,4-тетрагідропіримідин-4-іл)оцтової кислоти за допомогою перегрупування Курціуса відповідних ацилазидів, утворених *in situ* за обробки кислот дифенілфосфорилазидом. Визначено, що одержані продукти виявляють значний інгібувальний ефект на ізоформи PARP-1 і PARP-2 полі(АДФ-рибоза)-полімераз. Аналізом взаємозв'язку між структурою та активністю доведено, що N1-арильний замісник має суттєве значення для селективності та високої активності до PARP-2, а *пара*-фторобензильна група є оптимальною для вираженого невибіркового інгібування PARP-1 та PARP-2.

Ключові слова: перегрупування Курціуса; гетероциклізація; трифлуорометильна група; піроло[3,4-*d*]піримідини; інгібітори полі(АДФ-рибоза)-полімерази

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Citation: Lukianov, O. O; Tkachuk, V. M.; Stepanova, D. S.; Gillaizeau, I.; Sukach, V. A. An efficient synthesis of PARP inhibitors ontaining a 4-trifluoromethyl substituted 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione scaffold. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 18–26.
https://doi.org/10.24959/ophcj.23.298727
Received: *5 November 2023*; **Revised:** *24 November 2023*; **Accepted:** *25 November 2023*

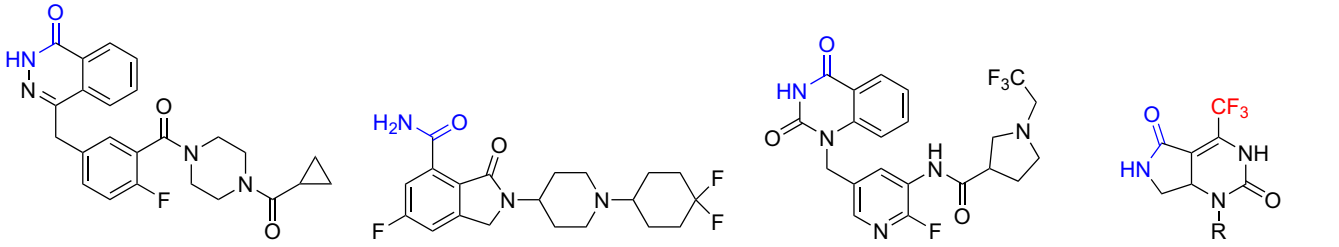
Funding: The authors received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

Poly(ADP-ribose) polymerase (PARP) has evolved as a promising molecular target in the cancer-targeted chemotherapy due to its pivotal role in restoring the genomic integrity [1]. The knowledge of its functions has led to the development of PARP inhibitors, which exert their anticancer activity by interrupting DNA repair mechanisms [2]. Several compounds (olaparib, niraparib, talazoparib, and rucaparib) have already been marketed for treating advanced ovarian cancer and breast cancer patients [3]. As a monotherapy, PARP inhibitors have been shown to selectively kill tumors harboring mutations or deletions in DNA repair genes related to homologous recombination pathways, such as BRCA-1 and BRCA-2. This phenomenon referred to as "synthetic lethality" has been successfully translated to clinical practice and now exemplifies a "personalized" approach to the cancer therapy [4].

A poly(ADP-ribose) polymerase family has 18 nuclear and cytoplasmic enzymes that cleave NAD+ to nicotinamide and ADP-ribose to form long and branched ADP-ribose polymers on target proteins, including topoisomerases, histones and PARP itself, and thus impact diverse cellular processes (replication, transcription, differentiation, gene regulation, protein degradation). In the family, PARP1 and PARP2 are the most attractive therapeutic targets for the anticancer drug development. In fact, the vast majority of PARP inhibitors developed to date demonstrate a lack of specificity for any given PARP isoform. This raises the challenging problem of developing small molecule PARP inhibitors with a high isoform selectivity as better-tolerated drugs [5]. PARP-1 accounts for more than 90% of the cellular PARP activity, thus representing a primarily targeted PARP isozyme for the therapeutic intervention. However, none of the currently FDA-approved PARP inhibitors selectively inhibits PARP-1. Moreover, olaparib (**Figure**), the most clinically successful drug, demonstrates superior (5-fold) inhibition of PARP-2 [6]. Compound NMS-P118 is one of the most PARP-1 selective clinical candidates (~150-fold selectivity over PARP-2) endowed with a pronounced anticancer activity in preclinical studies [7]. *Zhao et al.* identified compound **11a** with a nearly 40-fold PARP-2/PARP-1 selectivity [8].

Recently, we have reported that the 4-trifluoromethyl-substituted 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione core may serve as a scaffold for designing a novel class of PARP inhibitors, due primarily to the presence of the endocyclic amide fragment in the pyrrolidone ring, which resembles a key structural motif of the majority of PARP's nicotinamide site ligands [9]. This suggestion was supported by a molecular docking study, which additionally revealed that the trifluoromethyl group in position 4 of the novel scaffold may significantly contribute to the binding with the PARP enzyme NAD+ binding site due to the C···F orthogonal dipolar interaction with the backbone peptide fragment. As a result,



Olaparib (ref [6])
IC_{50} (PARP-1): 5 nM
IC_{50} (PARP-2): 1 nM

NMS-P118 (ref [7])
K_D (PARP-1): 0.03 μM
K_D (PARP-2): 3.93 μM

Compound 1 1a (ref [8])
IC_{50} (PARP-1): 467 nM
IC_{50} (PARP-2): 11.5 nM

Compounds 1a,b (ref [9])
a: R = PMP
IC_{50} (PARP-2): 0.46 μM
b: R = PFB
IC_{50} (PARP-2): 1.12 μM

Figure. Selected PARP-1 and PARP-2 inhibitors. PMP – *p*-methoxyphenyl; PFB – *p*-fluorobenzyl

compounds **1a,b** demonstrated a potent inhibition of the PARP-2 enzyme. In this work, we characterized a new series of 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione derivatives in the *in vitro* PARP-1 and PARP-2 radiometric assay to gain deeper insight into the inhibition selectivity of the isoforms and a preliminary structure-activity relationship.

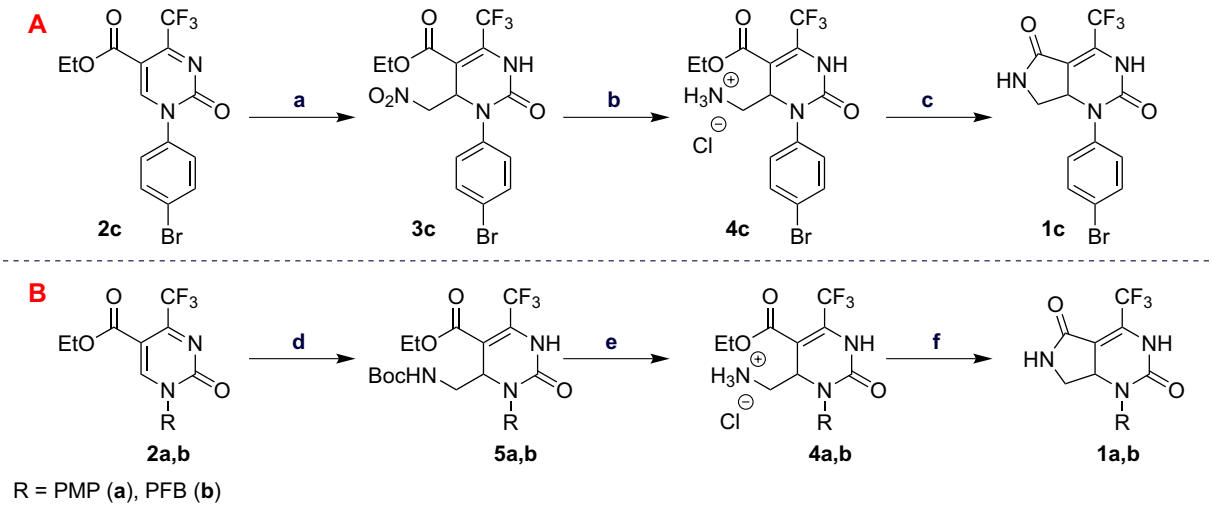
■ Results and discussion

Synthesis

The method previously developed for the preparation of 4-bromophenyl substituted 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione **1c** relied on regioselective nitromethane addition to ethyl carboxylate **2c**, chemoselective nitro group reduction in the adduct **3c** and subsequent intramolecular cyclocondensation of the resulting amine intermediate **4c** (**Scheme 1**, A) [10]. The main drawbacks of this approach were the low thermal stability of nitro compound **3c**, which easily underwent a retro nitro-Michael reaction to the starting reactants, and facile saturation of the C=C double bond in the 1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione system under a number of reducing conditions tested for step ***b***. It was found that reduction of the nitro group with zinc dust in acetic acid furnished amino ester hydrochloride **4c** in an acceptable 60% yield. Finally, compound **1c** was obtained in 83% yield after the treatment with aqueous NaOH at room temperature. This method is unsuitable for synthesizing N1-alkyl substituted derivatives due to an insufficient reactivity of N1-alkyl pyrimidones **2** in the nitromethane addition step.

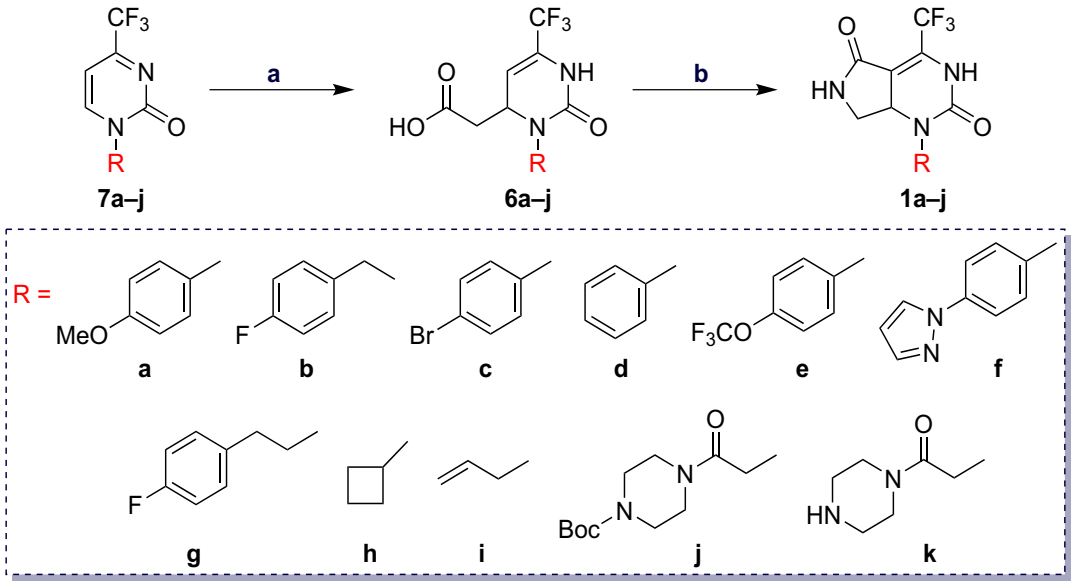
The second approach (**Scheme 1**, B) was based on the visible-light-mediated hydroaminoalkylation of pyrimidin-2(1*H*)-ones **2a,b** by the *aza*-Giese-type reaction with potassium *N*-Boc-aminomethyltrifluoroborate in the presence of an acridinium dye as a photocatalyst [9]. Further transformation of the radical addition products **5a,b** enabled the synthesis of amine hydrochlorides **4a,b** and, eventually, 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-dione derivatives **1a,b**, which showed promise as inhibitors of poly-(ADP-ribose) polymerase (PARP) enzymes (IC_{50} 0.46–1.12 μM for PARP-2 in a fluorometric assay). The method requires chromatographic purification of intermediates **5** due to the formation of by-products derived from the radical side reactions.

With the aim of developing a practical general approach to the desired heterocyclic system **1**, lacking the above-mentioned limitations, we used stable (2-oxo-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid derivatives **6a–j** (**Scheme 2**) as key intermediates in this work. Latter compounds were obtained in high yields from readily available pyrimidin-2(1*H*)-ones **7a–j** according to our reported procedure based on the regioselective decarboxylative malonic acid addition [11]. As found out, acids **6** can be easily converted into target compounds **1** in a single step by the interaction with diphenylphosphoryl azide and triethylamine under heating in toluene. The reaction proceeds *via* the Curtius rearrangement of the corresponding



Scheme 1. Previously reported methods for the preparation of compounds **1a–c**. *Reagents and conditions*: (**a**) $MeNO_2$ (5 equiv), Et_3N (0.1 equiv), CH_2Cl_2, 0–5 °C, 10 h, 70% yield; (**b**) 1. Zn, AcOH, 25 °C, 8 h; 2. 4M HCl in dioxane, 60% yield; (**c**) NaOH, MeOH/H_2O, 25 °C, 2 h, 83% yield; (**d**) $BocNHCH_2BF_3K$ (1.5 equiv), acridinium photocatalyst (3 mol%), acetone/MeOH (5:1), blue light irradiation, 25 °C, 16 h, 59–64% yield; (**e**) 1M HCl in dioxane, 25 °C, 6 h, 77–86% yield; (**f**) NaOH, EtOH, 60 °C, 6 h, 71–76% yield.
Boc – *tert*-butyloxycarbonyl; PFB – *p*-fluorobenzyl; PMP – *p*-methoxyphenyl

ISSN 2308-8303 (Print) / 2518-1548 (Online)



Scheme 2. The approach elaborated to 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-diones. *Reagents and conditions*: (**a**) malonic acid (5 equiv), Et_3N (1 equiv), DMSO, 80 °C 18 h, 59–79 % yield; (**b**) $(PhO)_2PON_3$ (1.2 equiv), Et_3N (1.5 equiv), toluene, 80 °C, 6 h, 42–63 % yield. Boc – *tert*-butyloxycarbonyl

in situ generated acyl azides and the subsequent intramolecular cyclocondensation of the resulting isocyanates to 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-diones. Thus, the target compounds **1a–j** featuring diverse N1-alkyl and N1-aryl substituents were obtained in 42–63% yields. The removal of the *N*-Boc-protection in product **1j** led to piperazine-containing compound **1k** in 87% yield. None of the synthetic steps in the novel approach required the chromatographic purification of both the intermediate and final products.

Biological activity

The results of studying the inhibitory activity for compounds **1a–i,k** against PARP-1 and PARP-2 enzymes are presented in **Table** (represented as the residual enzyme activity after the incubation with the inhibitors in the concentration of 10 μM). The results of the radiometric PARP inhibitory assay revealed that the N1-aryl-substitution within the 3,6,7,7a-tetrahydro-1*H*-pyrrolo-[3,4-d]pyrimidine-2,5-dione system (compounds **1a,c–f**) led to the complete PARP-2 inhibition in this concentration, while the activity of PARP-1 enzyme was in the range of 23.85–50.21%. The substitution by a N1-*p*-fluorobenzyl group gave rise to a potent and merely isoform non-selective inhibitor (compound **1b**). When R is a N1-*p*-fluorophenethyl group, compound **1g** exhibited a moderate selectivity and a good potency (up to 1.22% and 17.48% of the remaining activity of PARP-2 and PARP-1, respectively). The introduction of simple N1-alkyl groups, such as cyclobutyl or allyl substituents, in compounds **1h,i**, or a piperazine amide fragment in compound **1k** resulted in a weaker non-selective inhibition with 8.59–35.2% of the remaining enzyme activity.

Table. The remaining enzyme activity (%, n = 2: data 1 and data 2, relative to DMSO control) after the incubation of 10 μM of compounds **1a–i,k** with PARP-1 and PARP-2

Compound	PARP-1 activity (data 1; data 2), %	PARP-2 activity (data 1; data 2), %
1a	50.21; 49.18	6.92; 6.51
1b	6.29; 6.72	-2.62; 0.21
1c	33.57; 30.56	-2.08; 1.08
1d	41.64; 39.22	5.11; 5.87
1e	40.82; 39.32	3.90; 3.79
1f	25.05; 23,85	-3.20; -0.95
1g	17.48; 14.35	2.08; 1.22
1h	26.9; 26.3	21.22; 19.3
1i	20.81; 19.84	9.58; 8.59
1k	35.2; 33.27	18.27; 17.89

■ Conclusions

A series of 3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-*d*]pyrimidine-2,5-diones has been synthesized using a novel practical approach, and their inhibitory activity against PARP-1 and PARP-2 isoforms has been evaluated. Among all the compounds tested, derivatives **1a–g** have exhibited a potent enzyme inhibitory activity with the complete inhibition at a concentration of 10 μM, and **1a,d-f** have demonstrated specificity to PARP-2 (up to 41.64 of the remaining PARP-1 activity at the same concentration). These findings can provide promising structural chemotypes for the development of novel PARP inhibitors with a high potency and specificity.

Experimental part

Chemistry, general experimental information

All chemicals were obtained from Enamine LTD and used without further purification. Solvents were purified according to the standard procedures. Melting points were uncorrected. ^{19}F NMR, ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury-400 (^{13}C: 101 MHz; ^{19}F: 376 MHz) or Bruker Avance DRX-500/600 (^{13}C: 126 MHz, 151 MHz; ^{19}F: 470 MHz) or Mercury+ 300 Varian (^{13}C: 76 MHz, 302 MHz; ^{19}F: 188 MHz) spectrometers with TMS or CCl_3F as an internal standard. Multiplets were assigned as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *m* (multiplet) and *br. s* (broad singlet). Mass spectrometric detection of samples was performed with an Infinity 1260 UHPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a 6224 Accurate Mass TOF LC/MS system (Agilent Technologies, Singapore). Compounds **6b,i** and **7a–f,i** were prepared according to the literature procedures [11].

Biological assay conditions and protocol

The research was performed by the Reaction Biology Corporation according to the standard assay protocol recommended by the developer (https://www.reactionbiology.com/services/target-specific-assays/parp-assays). The reaction buffer was 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1% DMSO, and 20 μg/mL activated DNA. The ^{32}P-NAD$^+$ ([adenylate-^{32}P]-Nicotinamide Adenine Dinucleotide) concentration was 10 μM. Compounds were tested in a single-dose duplicate mode in a concentration of 10 μM. The control compound, PJ34, was tested in a 10-dose IC_{50} mode with a 3-fold serial dilution starting at 10 μM. The resulting IC_{50} values were in accordance with the published data: 13.5 nM (PARP-1) and 9.5 nM (PARP-2). PARP substrates were core histones (PARP-1) and histone H3.3 (PARP-2).

The substrate was prepared in the Reaction Buffer freshly obtained. The indicated PARP was delivered into the substrate solution and gently mixed. Compounds were delivered in DMSO into the reaction mixture using an Acoustic Technology (Echo 550, LabCyte Inc. Sunnyvale, CA) in the nanoliter range and incubated 20 min at room temperature. ^{32}P-NAD$^+$ was delivered into the reaction mixture to initiate the reaction. The mixture was incubated for 2 h at room temperature and then delivered to filter-paper and washed with 0.75% phosphoric acid for detection.

The data were analyzed using the Excel and GraphPad Prism software. The PARP activity data were expressed as the percent of the remaining PARP activity in test samples (data 1 and data 2) compared to vehicle (dimethyl sulfoxide) reactions. IC_{50} values and curve fits were obtained using the Prism4 Software (GraphPad).

The synthesis of compounds 1a-j

To a solution of compounds **6a–j** (1 mmol) and diphenylphosphoryl azide (0.33 g, 1.2 mmol) in toluene (10 mL), triethylamine (0.15 g, 1.5 mmol) was added. The mixture was stirred at 80 °C for 6 h. After completion of the reaction, the mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The residue obtained was treated with saturated solution of sodium hydrogen carbonate (20 mL). The solid was filtered, washed with water (20 mL), methyl *tert*-butyl ether (10 mL), dried on air and purified by recrystallization from acetonitrile to obtain the corresponding product as a white solid.

1-(4-Methoxyphenyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1a**)

Yield – 0.18 g (56%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.94 (1H, t, J = 8.5 Hz), 3.20 (1H, t, J = 8.8 Hz), 5.26 (1H, s), 3.76 (3H, s), 6.94 (2H, d, J = 8.4 Hz), 7.27 (2H, d, J = 8.4 Hz), 8.16 (1H, s), 10.10 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.8, 55.7, 55.8, 110.0, 114.5, 119.5 (q, J = 275.8 Hz), 127.6 (q, J = 38.4 Hz), 128.6, 131.6, 153.2, 158.5, 164.1. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –62.1 (s). HRMS (ESI): m/z calcd for $C_{14}H_{13}F_3N_3O_3$ $[M+H]^+$ 328.0904, found 328.0906.

1-(4-Fluorobenzyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1b**)

Yield – 0.23 g (62%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.15 (1H, t, J = 8.3 Hz), 3.54 (1H, t, J = 8.5 Hz), 4.22 (1H, d, J = 15.4 Hz), 4.51–4.55 (1H, m), 4.68 (1H, d, J = 15.5 Hz), 7.17 (2H, t, J = 8.7 Hz), 7.38 (2H, dd, J = 8.4, 5.5 Hz), 8.17 (1H, s), 9.97 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.7, 46.5, 54.5, 109.2, 115.8 (d, J = 21.4 Hz), 120.4 (q, J = 276.1 Hz), 127.4 (q, J = 38.2 Hz), 130.4 (d, J = 8.2 Hz), 133.3 (d, J = 3.0 Hz), 153.6, 162.0 (d, J = 243.2 Hz), 164.2. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –115.2 (t, J = 7.4 Hz, 1F), –61.8 (s, 3F). HRMS (ESI): m/z calcd for $C_{14}H_{12}F_4N_3O_2$ $[M+H]^+$ 330.0860, found 330.0855.

1-(4-Bromophenyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1c**)

Yield – 0.21 g (57%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.85–2.94 (1H, m),

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3.25–3.31 (1H, m), 5.34 (1H, br. s), 7.32 (2H, d, J = 7.0 Hz), 7.59 (2H, d, J = 7.6 Hz), 8.22 (1H, s), 10.23 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.1, 54.6, 109.7 (d, J = 2.6 Hz), 119.0 (q, J = 276.3 Hz), 119.5, 126.9 (q, J = 38.8 Hz), 128.8, 131.6, 137.7, 152.3, 163.3. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –61.55 (s). HRMS (ESI): m/z calcd for $C_{13}H_9BrF_3N_3O_2$ $[M+H]^+$ 375.9903, found 375.9900.

1-Phenyl-4-(trifluoromethyl)-4a,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1d**)

Yield – 0.18 g (61%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.89 (1H, t, J = 8.2 Hz), 3.28 (1H, t, J = 8.7 Hz), 5.31 – 5.39 (1H, m), 7.25–7.45 (5H, m), 8.19 (1H, s), 10.17 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.8, 55.3, 110.1, 119.5 (q, J = 275.7 Hz), 127.2, 127.4, 127.6 (q, J = 39.8 Hz), 129.3, 138.9, 153.0, 164.0. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –61.6 (s). HRMS (ESI): m/z calcd for $C_{13}H_{11}F_3N_3O_2$ $[M+H]^+$ 298.0798, found 298.0796.

1-(4-(Trifluoromethoxy)phenyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1e**)

Yield – 0.24 g (63%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.91 (1H, t, J = 8.2 Hz), 3.33 (1H, t, J = 8.5 Hz), 5.36 (1H, br. s), 7.39 (2H, d, J = 8.5 Hz), 7.49 (2H, d, J = 8.8 Hz), 8.20 (1H, s), 10.23 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.6, 55.1, 110.1 (d, J = 2.4 Hz), 119.4 (q, J = 276.0 Hz), 120.4 (q, J = 256.3 Hz), 121.8, 127.4 (q, J = 38.5 Hz), 128.9, 137.8, 147.0 (d, J = 1.9 Hz), 152.9, 163.8. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –61.6 (s, 1F), –56.9 (s, 1F). HRMS (ESI): m/z calcd for $C_{14}H_{10}F_6N_3O_3$ $[M+H]^+$ 382.0621, found 382.0625.

1-(4-(1H-Pyrazol-1-yl)phenyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1f**)

Yield – 0.19 g (52%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.91–2.99 (1H, m), 3.31–3.39 (2H, m), 5.37 (1H, br. s), 6.55 (1H, t, J = 2.2 Hz), 7.47 (2H, d, J = 8.4 Hz), 7.75 (1H, d, J = 1.7 Hz), 7.85 (2H, d, J = 8.4 Hz), 8.13 (1H, s), 8.49 (1H, d, J = 2.2 Hz), 10.11 (1H, s). ^{13}C NMR (76 MHz, DMSO-d_6), δ, ppm: 45.3, 54.9, 109.7, 117.9, 118.2, 119.1 (q, J = 276.1 Hz), 126.2, 127.1 (q, J = 38.5 Hz), 127.8, 135.9, 138.3, 141.9 (d, J = 2.8 Hz), 152.6, 163.6. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –62.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{13}F_3N_5O_2$ $[M+H]^+$ 364.1016, found 364.1021.

1-(4-Fluorophenethyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1g**)

Yield – 0.21 g (61%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.59–3.00 (2H, m), 3.00–3.24 (2H, m), 3.49–3.81 (2H, m), 4.70 (1H, s), 6.91–7.22 (2H, m), 7.22–7.50 (2H, m), 8.19 (1H, s), 9.82 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 32.0, 45.6, 54.2, 109.0, 115.5 (d, J = 21.0 Hz), 119.4 (q, J = 275.9 Hz), 127.4 (q, J = 37.9 Hz), 131.0, 131.1, 135.6, 153.2, 161.4 (d, J = 242.7 Hz), 164.2. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –116.8 (s, 1F), –61.8 (s, 3F). HRMS (ESI): m/z calcd for $C_{15}H_{14}F_4N_3O_2$ $[M+H]^+$ 344.1017, found 344.1011.

1-Cyclobutyl-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1h**)

Yield – 0.13 g (47%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.45–1.75 (2H, m), 2.03–2.10 (2H, m), 2.39–2.47 (2H, m), 3.20 (1H, t, J = 8.4 Hz), 3.72 (1H, t, J = 8.4 Hz), 4.04 (1H, p, J = 8.9 Hz), 4.74 (1H, s), 8.16 (1H, s), 9.64 (1H, s). ^{13}C NMR (101 MHz, DMSO-d_6), δ, ppm: 15.1, 28.3, 28.4, 47.3, 51.1, 54.9, 108.8, 119.4 (q, J = 276.2 Hz), 127.2 (q, J = 38.4 Hz), 153.0, 164.1. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –62.2 (s). HRMS (ESI): m/z calcd for $C_{11}H_{13}F_3N_3O_2$ $[M+H]^+$ 276.0954, found 276.0959.

1-Allyl-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1H-pyrrolo[3,4-d]pyrimidine-2,5-dione (**1i**)

Yield – 0.11 g (42%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.21 (1H, t, J = 8.3 Hz), 3.53–3.63 (2H, m), 4.10 (1H, dd, J = 15.7, 5.0 Hz), 4.65 (1H, dq, J = 7.8, 4.1 Hz), 5.20 (1H, d, J = 10.2 Hz), 5.27 (1H, d, J = 17.1 Hz), 5.72–5.86 (1H, m), 8.17 (1H, s), 9.86 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 45.6, 46.3, 54.2, 109.3, 118.9, 119.4 (q, J = 276.3 Hz), 127.4 (d, J = 38.5 Hz), 133.0, 153.2, 164.2. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –62.3 (s). HRMS (ESI): m/z calcd for $C_{11}H_{11}F_3N_3O_2$ $[M+H]^+$ 262.0798, found 262.0802.

tert-Butyl 4-(2-(2,5-dioxo-4-(trifluoromethyl)-2,3,5,6,7,7a-hexahydro-1H-pyrrolo[3,4-d]pyrimidin-1-yl)acetyl)piperazine-1-carboxylate (**1j**)

Yield – 0.28 g (63%). M. p. >250 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.41 (9H, s), 3.15 (1H, t, J = 8.4 Hz), 3.38 (8H, q, J = 19.9 Hz), 3.61 (1H, t, J = 8.5 Hz), 3.77 (1H, d, J = 17.0 Hz), 4.40 (1H, d, J = 17.0 Hz), 4.74–4.83 (1H, m), 8.17 (1H, s), 9.88 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 28.5, 41.6, 44.3, 44.8, 45.6, 55.0, 79.7, 109.3, 119.5 (q, J = 276.0 Hz), 127.4 (q, J = 38.6 Hz), 153.8, 154.3, 164.2, 166.3. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –61.8 (s). HRMS (ESI): m/z calcd for $C_{18}H_{25}F_3N_5O_5$ $[M+H]^+$ 448.1802, found 448.1805.

The synthesis of 1-(2-oxo-2-(piperazin-1-yl)ethyl)-4-(trifluoromethyl)-3,6,7,7a-tetrahydro-1*H*-pyrrolo[3,4-d]pyrimidine-2,5-dione hydrochloride (1k)

Compound **1j** (0.2 g, 0.45 mmol) was treated with 4M HCl in dioxane (5 mL), and the resulting

mixture was stirred at room temperature for 6 h. Methyl *tert*-butyl ether (10 mL) was added to the mixture. The solid was collected by filtration and washed with methyl *tert*-butyl ether (2×10 mL) to give compound **1k**.

A beige solid. Yield – 0.15 g (87%). M. p. >250 °C. ^{1}H NMR (302 MHz, DMSO-d_6), δ, ppm: 2.97–3.19 (5H, m), 3.57–3.73 (5H, m), 3.82 (1H, d, J = 17.1 Hz), 4.44 (1H, d, J = 17.0 Hz), 4.68–4.82 (1H, m), 8.23 (1H, s), 9.60 (2H, br. d, J = 29.5 Hz), 9.90 (1H, s). ^{13}C NMR (76 MHz, DMSO-d_6), δ, ppm: 41.1, 42.4 (d, J = 7.1 Hz), 44.3, 45.3, 54.6, 109.0 (d, J = 2.3 Hz), 119.0 (q, J = 276.1 Hz), 126.9 (q, J = 38.3 Hz), 153.4, 163.8, 165.9. ^{19}F NMR (188 MHz, DMSO-d_6), δ, ppm: –61.7 (s). HRMS (ESI): m/z calcd for $C_{13}H_{17}F_3N_5O_3$ $[M+H]^+$ 348.1278, found 348.1280.

The synthesis of compounds 6a,c–h,j

To the solution of compounds **7a,c–h,j** (3 mmol) and malonic acid (1.56 g, 15 mmol, 5 equiv) in DMSO (10 mL), triethylamine (0.3 g, 3 mmol) was added. The mixture was stirred at 80 °C for 18 h. After completion of the reaction, the mixture was cooled, diluted with 0.4M hydrochloric acid (20 mL), and the product was extracted with dichloromethane (3×20 mL). The combined organic layers were washed with brine (2×20 mL), dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue obtained was crystallized from hexane/methyl *tert*-butyl ether (1:5) to give the corresponding product as a white solid.

2-(3-(4-Methoxyphenyl)-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6a**)

Yield – 0.75 g (76%). M. p. 194–195 °C. ^{1}H NMR (302 MHz, DMSO-d_6), δ, ppm: 2.46 (2H, d, J = 5.9 Hz), 3.76 (3H, s), 4.56–4.77 (1H, m), 5.53–5.77 (1H, m), 6.95 (2H, d, J = 8.4 Hz), 7.26 (2H, d, J = 8.4 Hz), 9.60 (1H, s), 12.41 (1H, s). ^{13}C NMR (76 MHz, DMSO-d_6), δ, ppm: 38.43, 55.31, 56.19, 102.57 (d, J = 5.5 Hz), 114.20, 119.86 (q, J = 272.3 Hz), 126.99 (q, J = 34.6 Hz), 132.88, 129.63, 151.52, 157.96, 171.21. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –69.7 (s). HRMS (ESI): m/z calcd for $C_{14}H_{14}F_3N_2O_4$ $[M+H]^+$ 331.0900, found 331.0897.

2-(3-(4-Bromophenyl)-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6c**)

Yield – 0.79 g (69%). M. p. 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.47 (2H, d, J = 5.8 Hz), 4.85 (1H, br. s, J = 6.6 Hz), 5.64 (1H, br. s), 7.35 (2H, d, J = 8.1 Hz), 7.59 (2H, d, J = 8.2 Hz), 9.72 (1H, s), 12.42 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 38.4, 55.7, 102.9 (d, J = 5.2 Hz), 119.6, 119.8 (q, J = 272.1 Hz), 126.9 (q, J = 34.9 Hz), 130.4, 131.8, 139.6, 151.1, 171.0. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –69.6 (s). HRMS (ESI): m/z calcd for $C_{13}H_{11}BrF_3N_2O_3$ $[M+H]^+$ 379.9900, found 378.9895.

2-(2-Oxo-3-phenyl-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6d**)

Yield – 0.71 g (79%). M. p. 116–117 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.42–2.49 (2H, m), 4.82 (1H, d, J = 6.3 Hz), 5.63 (1H, d, J = 4.8 Hz), 7.30 (1H, t, J = 7.2 Hz), 7.33–7.46 (4H, m), 9.66 (1H, s), 12.45 (1H, s). ^{13}C NMR (126 MHz, chloroform-d), δ, ppm: 38.8, 56.3, 103.2 (d, J = 5.2 Hz), 120.3 (q, J = 272.4 Hz), 127.3 (d, J = 14.1 Hz), 127.4, 128.7, 129.4, 140.7, 151.8, 171.5. ^{19}F NMR (376 MHz, DMSO-d_6): δ –69.6 (s). HRMS (ESI): m/z calcd for $C_{13}H_{12}F_3N_2O_3$ $[M+H]^+$ 301.0795, found 301.0799.

2-(2-Oxo-3-(4-(trifluoromethoxy)phenyl)-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl) acetic acid (**6e**)

Yield – 0.82 g (71%). M. p. 176–177 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.47 (2H, d, J = 5.8 Hz), 4.79–4.93 (2H, m), 5.60–5.70 (1H, m), 7.40 (2H, d, J = 8.5 Hz), 7.51 (2H, d, J = 8.5 Hz), 9.74 (1H, s), 12.42 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 38.31, 55.81, 102.85 (d, J = 5.1 Hz), 119.76 (q, J = 272.1 Hz), 120.07 (q, J = 256.3 Hz), 121.48, 126.90 (q, J = 35.0 Hz), 130.17, 139.30, 146.63, 151.24, 171.02. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –69.6 (s, 1F), –57.4 (s, 1F). HRMS (ESI): m/z calcd for $C_{14}H_{11}F_6N_2O_4$ $[M+H]^+$ 385.0618, found 385.0620.

2-(3-(4-(1H-Pyrazol-1-yl)phenyl)-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl) acetic acid (**6f**)

Yield – 0.68 g (62%). M. p. 204–205 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.46–2.52 (2H, m), 4.86–4.90 (1H, m), 5.62–5.68 (1H, m), 6.56 (1H, t, J = 2.2 Hz), 7.49 (2H, d, J = 8.6 Hz), 7.76 (1H, d, J = 1.7 Hz), 7.87 (2H, d, J = 8.4 Hz), 8.52 (1H, d, J = 2.5 Hz), 9.72 (1H, br. s), 12.43 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 38.93, 56.38, 108.43, 119.25, 103.29 (d, J = 5.1 Hz), 120.27 (q, J = 272.1 Hz), 127.38 (q, J = 34.9 Hz), 128.32, 129.83, 138.54, 138.62, 141.58, 151.78, 171.58. ^{19}F NMR (376 MHz, DMSO-d_6): δ –69.6 (s). HRMS (ESI): m/z calcd for $C_{16}H_{14}F_3N_4O_3$ $[M+H]^+$ 367.1013, found 367.1010.

2-(3-(4-Fluorophenethyl)-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6g**)

Yield – 0.72 g (69%). M. p. 132–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 2.45 (1H, d, J = 7.8 Hz), 2.63 (1H, dd, J = 15.9, 4.8 Hz), 2.73 (1H, dt, J = 13.8, 6.7 Hz), 2.78–2.89 (1H, m), 3.05–3.17 (1H, m), 3.73–3.85 (1H, m), 4.24–4.34 (1H, m), 5.45 (1H, d, J = 5.3 Hz), 7.08 (2H, t, J = 8.7 Hz), 7.24

ISSN 2308-8303 (Print) / 2518-1548 (Online)

(2H, dd, J = 8.3, 5.5 Hz), 9.39 (1H, s), 12.48 (1H, s). ^{13}C NMR (126 MHz, chloroform-*d*), δ, ppm: 33.0, 46.5, 52.6, 102.8, 115.4 (d, J = 20.9 Hz), 121.3 (q), 127.7 (q, J = 34.7 Hz), 131.0 (d, J = 7.9 Hz), 135.6, 152.6, 160.4, 162.3, 172.0. ^{19}F NMR (470 MHz, DMSO-d_6), δ, ppm: –117.1 (s, 1F), –69.0 (s, 3F). HRMS (ESI): m/z calcd for $C_{15}H_{15}F_4N_2O_3$ $[M+H]^+$ 347.1013, found 347.1010.

2-(3-Cyclobutyl-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6h**)

Yield – 0.49 g (59%). M. p. 157–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.49–1.65 (2H, m), 2.04–2.23 (5H, m), 2.32–2.46 (1H, m), 4.08 (1H, p, J = 8.4 Hz), 4.34 – 4.43 (1H, m), 5.56 (1H, d, J = 6.0 Hz), 9.37 (1H, s), 12.25 (1H, s). ^{13}C NMR (126 MHz, chloroform-*d*), δ, ppm: 14.7, 27.7, 30.0, 49.6, 50.8, 103.5, 120.3 (q, J = 272.3 Hz), 127.9 (q, J = 34.9 Hz), 152.7, 171.7. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –69.3 (s). HRMS (ESI): m/z calcd for $C_{11}H_{14}F_3N_2O_3$ $[M+H]^+$ 279.0951, found 279.0955.

2-(3-(2-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-2-oxoethyl)-2-oxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidin-4-yl)acetic acid (**6j**)

Yield – 0.88 g (65%). M. p. 221–222 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.41 (9H, s), 2.47 (1H, d, J = 16.2 Hz), 2.73 (1H, d, J = 16.1 Hz), 3.20–3.40 (8H, m), 3.99 (1H, d, J = 16.8 Hz), 4.30 – 4.35 (1H, m), 4.40 (1H, d, J = 16.6 Hz), 5.47 (1H, s), 9.36 (1H, s), 12.40 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 28.5, 41.7, 44.2, 46.4, 53.7, 79.6, 102.7, 102.8, 120.2 (q, J = 272.2 Hz), 127.2 (q, J = 34.5 Hz), 152.6, 154.3, 167.0, 172.1. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –69.6 (s). HRMS (ESI): m/z calcd for $C_{18}H_{26}F_3N_4O_6$ $[M+H]^+$ 451.1799, found 451.1795.

The synthesis of compounds 7g,h

4-Ethoxy-1,1-difluorobut-3-en-2-one (1.5 g, 10 mmol) and the corresponding *N*-alkylurea (10 mmol) were dissolved in methanol (15 mL), then concentrated hydrochloric acid was added (5 mL). The mixture was refluxed for 12 h. After cooling, the solution was evaporated to the half volume, and then water (10 mL) was added. The precipitate formed was filtered, washed with water (10 mL), diethyl ether (10 mL) and dried on air.

1-(4-Fluorophenethyl)-4-(trifluoromethyl)pyrimidin-2(1H)-one (**7g**)

Using 1-(4-fluorophenethyl)urea (1.82 g, 10 mmol). A white solid. Yield – 1.91 g (67%). M. p. 183–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 3.01 (2H, t, J = 7.4 Hz), 4.16 (2H, t, J = 7.4 Hz), 6.81 (1H, d, J = 6.6 Hz), 7.12 (2H, t, J = 8.9 Hz), 7.20–7.29 (2H, m), 8.38 (1H, d, J = 6.6 Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 33.0, 52.9, 99.1, 115.7 (d, J = 21.2 Hz), 120.0 (q, J = 277.2 Hz), 131.2 (d, J = 8.0 Hz), 134.0 (d, J = 3.3 Hz), 154.7, 154.9, 161.5 (q, J = 35.4 Hz), 161.6 (d, J = 242.3 Hz). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –116.7 (s, 1F), –70.9 (s, 3F). HRMS (ESI): m/z calcd for $C_{13}H_{11}F_4N_2O$ $[M+H]^+$ 287.0802, found 287.0806.

1-Cyclobutyl-4-(trifluoromethyl)pyrimidin-2(1H)-one (**7h**)

Using 1-cyclobutylurea (1.14 g, 10 mmol). A white solid. Yield – 1.33 g (61%). M. p. 103–105 °C. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.60–1.76 (2H, m), 1.76–2.05 (4H, m), 4.30–4.59 (1H, m), 6.83 (1H, d, J = 6.6 Hz), 8.32 (1H, d, J = 6.6 Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 17.46, 31.49, 54.40, 104.60 (d, J = 11.0 Hz), 120.69 (q, J = 271.7 Hz), 153.64, 160.90 (q, J = 34.7 Hz). ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –70.1 (s). HRMS (ESI): m/z calcd for $C_9H_{10}F_3N_2O$ $[M+H]^+$ 219.0740, found 219.0736.

The synthesis of *tert*-butyl 4-(2-(2-oxo-4-(trifluoromethyl)pyrimidin-1(2*H*)-yl)acetyl)piperazine-1-carboxylate (7j)

To an ice-cooled stirred solution of 4-(trifluoromethyl)pyrimidin-2(1*H*)-one (1.64 g, 10 mmol, 1 equiv) in anhydrous DMF (20 mL), potassium *tert*-butoxide (1.23 g, 11 mmol, 1.1 equiv) was added in one portion, and the mixture was stirred at 0 °C for 15 min. Afterwards, *tert*-butyl 4-(2-chloroacetyl)piperazine-1-carboxylate (2.63 g, 10 mmol, 1 equiv) was added to the mixture. The reaction mixture was warmed to room temperature for over 1 h, stirred at room temperature overnight, and then poured on ice water. The solid was collected by filtration, washed with water (3×30 mL) and cyclohexane (30 mL), and dried on air.

A white solid. Yield – 2.89 g (74%). M. p. 253 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.42 (9H, s), 3.31–3.62 (8H, m), 4.95 (2H, s), 6.94 (1H, d, J = 6.6 Hz), 8.48 (1H, d, J = 6.6 Hz). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 28.5, 42.0, 44.5, 52.0, 79.8, 99.2, 120.0 (q, J = 277.6 Hz), 154.3, 154.8, 156.2, 162.0 (q, J = 35.5 Hz), 164.8. ^{19}F NMR (376 MHz, DMSO-d_6), δ, ppm: –70.2 (s). HRMS (ESI): m/z calcd for $C_{16}H_{22}F_3N_4O_4$ $[M+H]^+$ 391.1588, found 391.1581.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)

Journal of Organic and Pharmaceutical Chemistry

Original Research

OPEN ACCESS
Freely available online on
http://ophcj.nuph.edu.ua

UDC 547.775+547.722+577.152.1

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Inhibition of Xanthine Oxidase by Pyrazolone Derivatives Bearing a 4-(Furan-2-yl)benzoic Acid Moiety

Abstract

The pyrazolone-based 4-(furan-2-yl)benzoic acids have been synthesized and studied as xanthine oxidase inhibitors. This enzyme is one of the therapeutic targets for the treatment of hyperuricemia and related diseases. The compounds studied have found to exhibit low micromolar IC_{50} values relative to the enzyme *in vitro*, depending on substituents in position 3 of the pyrazolone ring. However, the inhibitory effects observed are reduced in the presence of bovine serum albumin or Tween-80. Among the pyrazolone derivatives synthesized, 4-(5-((3-methyl-5-oxo-1-phenyl-1,5-dihydro-4*H*-pyrazol-4-ylidene)methyl)furan-2-yl)benzoic acid has been found to be the most potent inhibitor of xanthine oxidase. Kinetic results have shown that this compound is a mixed-type inhibitor with higher affinity to the free enzyme than to the enzyme-substrate complex. The results of the molecular docking and molecular dynamics show that the carboxylic group of the inhibitor can form a salt bridge with Arg880 and a hydrogen bond with Thr1010. These interactions can be key factors in the enzyme-inhibitor complex stabilization.

Keywords: xanthine oxidase; inhibition; pyrazolone; benzoic acid; molecular docking; molecular dynamics

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Інгібування ксантиноксидази похідними піразолону, що містять фрагмент 4-(фуран-2-іл)бензойної кислоти

Анотація

4-(Фуран-2-іл)бензойні кислоти з фрагментом піразолону було синтезовано та досліджено як інгібітори ксантиноксидази. Цей ензим є однією з терапевтичних мішеней для лікування гіперурикемії та супутніх захворювань. Вивчені сполуки демонстрували низькомікромолярні значення IC_{50} щодо ензиму *in vitro*, залежно від замісників у положенні 3 піразолонового кільця. Однак спостережувані інгібувальні ефекти знижувались за наявності бичачого сироваткового альбуміну або твіну-80. Серед синтезованих похідних піразолону 4-(5-((3-метил-5-оксо-1-феніл-1,5-дигідро-4*H*-піразол-4-іліден)метил)фуран-2-іл)бензойна кислота виявилась найпотужнішим інгібітором ксантиноксидази. Кінетичні дослідження засвідчили, що ця сполука є інгібітором змішаного типу з більшою спорідненістю до вільного ензиму, ніж до ензим-субстратного комплексу. Результати молекулярного докінгу і молекулярної динаміки свідчать про те, що карбоксильна група інгібітора може формувати сольовий місток із залишком Arg880 і водневий зв'язок із залишком Thr1010. Ці взаємодії можуть бути ключовими факторами стабілізації комплексу ензим-інгібітор.

Ключові слова: ксантиноксидаза; інгібування; піразолони; бензойна кислота; молекулярний докінг; молекулярна динаміка

Citation: Beiko, A. V.; Kobzar, O. L.; Kachaeva, M. V.; Pilyo, S. G.; Tanchuk, V. Yu.; Vovk, A. I. Inhibition of xanthine oxidase by pyrazolone derivatives bearing a 4-(furan-2-yl)benzoic acid moiety. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 27–35.
https://doi.org/10.24959/ophcj.23.298726
Received: *1 October 2023*; **Revised:** *23 October 2023*; **Accepted:** *28 October 2023*

Funding: The work was founded by the National Academy of Sciences of Ukraine.
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

Xanthine oxidase (XO) is the main cytosolic molybdenum-containing enzyme of the purine catabolism that catalyzes the transformation of hypoxanthine to xanthine and uric acid. Increased xanthine oxidase activity is not only the cause of hyperuricemia and gout, but also the cause of hyperuricemia-related disorders, such as metabolic syndrome, renal and cardiovascular diseases, diabetes, hypertension, and cancer. In addition, the enzymatic reaction is accompanied by the formation of reactive oxygen species causing oxidative stress and systemic inflammation [1]. Currently, FDA-approved drugs, such as allopurinol and febuxostat (as well as topiroxostat approved in Japan), are widely used in the treatment of the diseases associated with the enhanced XO activity. However, these drugs possess significant adverse effects and are not suitable for a long-term treatment of asymptomatic hyperuricemia. Thus, the development of new XO inhibitors is needed [2]. For this reason, the derivatives of imidazole, pyrazole, thiazole, selenazole, isoxazole, pyrimidine, quinolone, purine, pyrazolopyrimidine, pyrazoloquinazoline, and some other heterocyclic compounds have been previously synthesized and studied as XO inhibitors [3]. Among them, compounds bearing the carboxyl group like febuxostat appeared to be effective inhibitors of the enzyme. The binding mechanisms of such compounds to the XO active site include the interactions of their carboxylate groups with Arg880 and Thr1011 [3].

The present study is aimed to synthesize the pyrazolone-based 4-(furan-2-yl)benzoic acids for their evaluation as XO inhibitors. It should be noted that structurally similar compounds namely pyrazolone-based phenylfuran-2-yl derivatives were described previously as inhibitors of SARS-CoV and MERS-CoV 3C-like protease [4], heptosyltransferase WaaC [5], and neuraminidase [6].

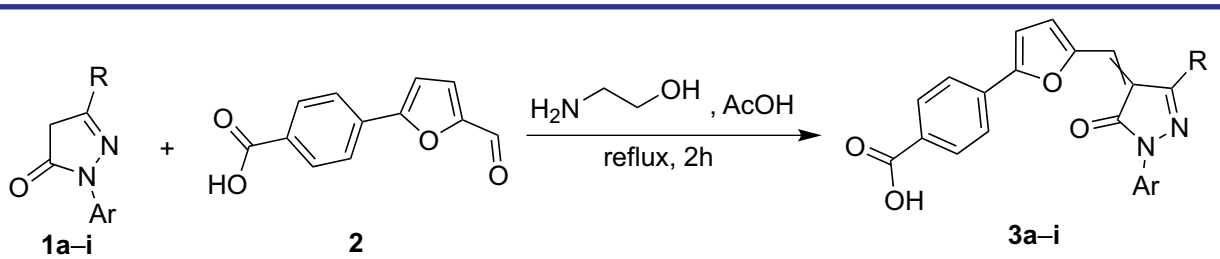
■ Results and discussion

Compounds **3a**–**i** were synthesized with a yield of 80–87% according to **Scheme** by the Knoevenagel condensation of pyrazolones **1** with 4-(5-formylfuran-2-yl)benzoic acid (**2**). The pyrazolones **1** bearing various substituents in positions 3 and 5 were obtained by the condensation of β-ketocarboxylic esters with hydrazine derivatives (Knorr synthesis) [7]. 4-(5-Formylfuran-2-yl)benzoic acid was obtained *via* the arylation of 2-furancarboxaldehyde by a diazonium salt of *p*-aminobenzoic acid (Meerwein arylation method) [8]. ^{1}H NMR, IR, and mass-spectra confirmed the structures of the compounds **3a**–**i** synthesized. In ^{1}H NMR spectra, the methyne proton (–CH=) signals appear in the range from 8.06 to 6.87 ppm, overlapping with the signals from the aryl substituents (except compound **3g** with the chemical shift of the methine proton singlet of 7.85 ppm). The ^{1}H NMR signals of the carboxylic groups were observed at 12.17–13.11 ppm. In the IR spectra, the intensive absorption bands of carbonyl groups were found at 1679–1696 cm^{-1}.

The synthesized compounds **3a**–**i** can exist as *E*- or *Z*-isomers. It was shown previously that (5-phenylfuran-2-yl)methylene-containing pyrazolone derivatives synthesized by the same route were a mixture of *E*-*Z*-isomers with a *Z*-isomer as a major product [5, 6].

The pyrazolone derivatives **3a**–**i** were studied *in vitro* as inhibitors of xanthine oxidase from bovine milk. This enzyme was characterized by 90% amino acid sequence identity to XO from the human liver [9]. The IC_{50} values determined from the dose-dependent curves of the enzyme inhibition (**Figure 1**) are presented in **Table 1**. These values are inhibitor concentrations, at which the XO activity was decreased by 50%.

According to the results obtained, compounds **3a**–**i** exhibited low micromolar IC_{50} values towards



1a, **3a**: Ar = Ph, R = Me
1b, **3b**: Ar = Ph, R = CF_3
1c, **3c**: Ar = Ph, R = Ph
1d, **3d**: Ar = Ph, R = 4-FC_6H_4
1e, **3e**: Ar = Ph, R = 4-ClC_6H_4
1f, **3f**: Ar = Ph, R = 4-$O_2NC_6H_4$
1g, **3g**: Ar = Ph, R = thiophen-2-yl
1h, **3h**: Ar = Ph, R = pyridin-4-yl
1i, **3i**: Ar = 4-MeC_6H_4, R = Ph

Scheme. The synthesis of pyrazolone-based 4-(furan-2-yl)benzoic acids **3a**–**i**

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Table 1. Pyrazolone-based 4-(furan-2-yl)benzoic acids as xanthine oxidase inhibitors*

Compound	IC_{50}, μM	IC_{50}, μM (in the presence of BSA)	IC_{50}, μM (in the presence of Tween-80)
3a	0.036 ± 0.0025	0.09 ± 0.02	0.11 ± 0.01
3b	0.52 ± 0.09	2.54 ± 0.69	2.44 ± 0.37
3c	0.096 ± 0.006	2.16 ± 0.48	3.74 ± 0.43
3d	0.15 ± 0.03	2.32 ± 0.10	2.55 ± 0.12
3e	0.17 ± 0.04	3.30 ± 0.68	7.48 ± 1.04
3f	0.22 ± 0.015	3.77 ± 0.09	7.88 ± 1.42
3g	0.058 ± 0.012	4.66 ± 0.91	2.58 ± 0.25
3h	0.22 ± 0.02	1.87 ± 0.51	2.20 ± 0.29
3i	0.06 ± 0.014	0.63 ± 0.18	2.08 ± 0.33
Febuxostat	0.0062 ± 0.0007	0.0075 ± 0.0001	0.0056 ± 0.0005

Note: *IC_{50} values were determined from 2-3 series of experiments and shown as an average value ± standard deviation. The concentrations of BSA or Tween-80 in the model systems were 2 μM and 0.025 vol. %, respectively

XO *in vitro*. Compound **3a** bearing 3-methyl-5-phenyl pyrazolone part had an IC_{50} value of 36 nM; it was the closest to that for the inhibition by febuxostat (**Table 1**) and significantly exceeded the IC_{50} value (4.5 μM) of 4-(5-formylfuran-2-yl) benzoic acid (**2**) observed. This indicates that the inhibition efficiency of compound **3a** was ensured due to a pyrazolone scaffold. The replacement of the methyl group in the structure of compound **3a** on the trifluoromethyl substituent (derivative **3b**) led to a 14-fold loss of the XO inhibition activity. Compounds **3c**–**h** with phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-nitrophenyl, thiophen-2-yl, or pyridin-4-yl substituents in position 3 of the pyrazolone ring, as well as compound **3i** bearing the 1-(4-tolyl)-3-phenyl-substituted pyrazolone moiety were characterized by low differences in the IC_{50} values ranging from 0.058 to 0.22 μM.

The inhibition effects of most of the compounds studied here were found to be significantly reduced (up to 80-fold) by bovine serum albumin (BSA) or Tween-80 (**Table 1**). The inhibitory activity in the presence of BSA and a detergent was less changed for compound **3a** bearing a methyl group at position 3 of the pyrazolone ring and decreased most strongly for 3-(thiophen-2-yl)-substituted compound **3g**. In contrast to this, BSA or Tween-80 almost did not change the inhibitory potency of febuxostat as a reference compound against XO.

Taking into account that the decrease of the XO inhibition can be related to the binding of the inhibitors to BSA, a spectral study was performed. It was found that compound **3i** quenched the fluorescence of BSA (**Figure 2**).

The BSA fluorescence spectra were measured at different temperatures (298 K, 303 K, 308 K, and 313 K) in the presence of 0.5 μM, 1 μM, 2 μM, and 3 μM of compound **3i**. The Stern-Volmer

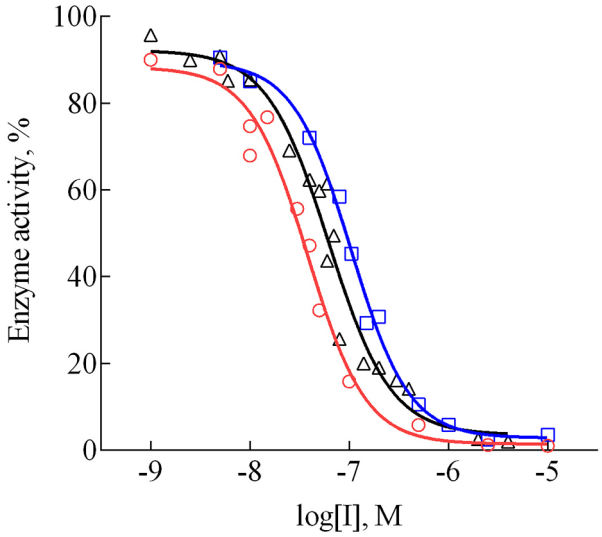


Figure 1. Dose-dependent curves of xanthine oxidase inhibition by compounds **3a** (○), **3c** (□) and **3i** (Δ)

quenching constants (K_{SV}) obtained from plots of F_0/F *vs.* the quencher concentration were 2.55×10^5 M^{-1} ($R^2 = 0.98$), 3.42×10^5 M^{-1} ($R^2 = 0.96$), 5.36×10^5 M^{-1} ($R^2 = 1.00$), 6.42×10^5 M^{-1} ($R^2 = 0.99$), respectively. The K_{SV} values increased with an increase in temperature. This dependence can indicate a dynamic quenching process. However, further analysis showed that the apparent bimolecular quenching rate constants (k_q) were three orders of magnitude larger than the value of the maximum scatter collision quenching constant of various quenchers with biopolymers (2.0×10^{10} M^{-1} sec^{-1}) [10]. The calculated values of k_q were 4.39×10^{13} M^{-1} sec^{-1}, 5.90×10^{13} M^{-1} sec^{-1}, 9.25×10^{13} M^{-1} sec^{-1}, and 1.11×10^{14} M^{-1} sec^{-1} for 298 K, 303 K, 308 K, and 313 K, respectively. Thus, the fluorescence quenching of BSA by compound **3i** can be also caused by the complex formation.

The results of kinetic studies were analyzed to elucidate the mechanism of the XO inhibition

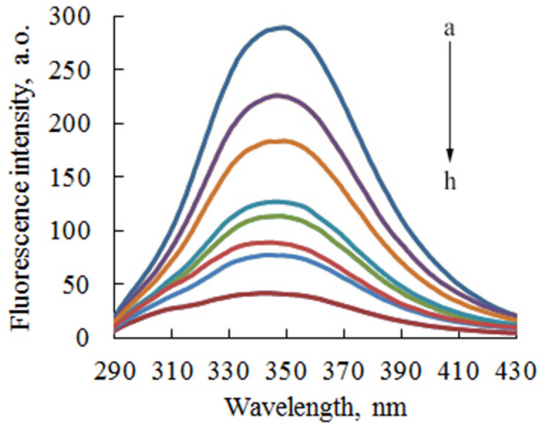


Figure 2. Fluorescence emission spectra of BSA in the absence (**a**) and presence (**b** → **h**) of compound **3i** measured at a temperature of 298 K, λ_{ex} of 280 nm, and slits of 5 nm. The BSA concentration was 2 μM. The concentrations of compound **3i** were 0 μM, 1 μM, 2 μM, 4 μM, 5 μM, 6 μM, 8 μM, and 10 μM

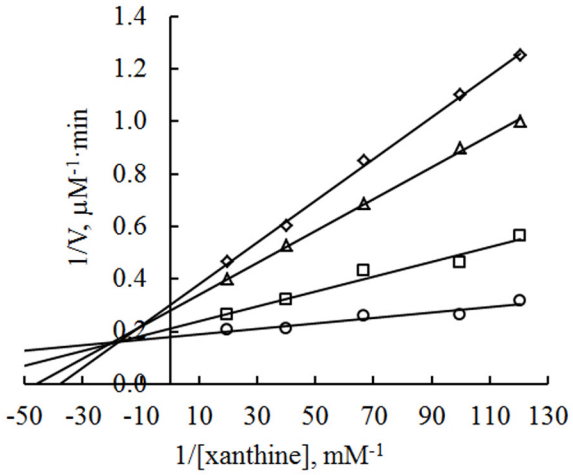


Figure 3. Lineweaver-Burke plots for the XO inhibition by compound **3a**. The inhibitor concentrations were 0 (○), 15 nM (□), 30 nM (Δ), and 45 nM (◊)

by pyrazolone derivative **3a**. The double reciprocal Lineweaver-Burk plots shown in **Figure 3** demonstrate that compound **3a** is a mixed-type inhibitor of the enzyme. The competitive and non-competitive inhibition constants (K_i and K_i') calculated were 7.11 ± 1.27 nM and 66.5 ± 15.4 nM, respectively. The K_i value being one order of magnitude lower than the K_i' value indicates that the affinity of the compound is higher to the free enzyme than to the enzyme-substrate complex.

Molecular docking calculations were carried out using the AutoDock Vina software [11]. The affinity of compound **3a** (*Z*-isomer) to XO was characterized by a docking energy of -10.8 kcal mol^{-1}. According to the results obtained, the 4-(furan-2-yl)benzoic acid fragment of compound **3a** is located in the substrate binding region near the molybdopterin cofactor. The carboxylic group of the inhibitor has a salt-bridge interaction with Arg880, as well as hydrogen bonds with Thr1010 and a water molecule (HOH1457), which interacts with Glu1261. The phenyl group of the 4-(furan-2-yl)benzoic acid part of the inhibitor forms face-to-face and edge-to-face π-π-stacking interactions with Phe914 and Phe1009. The furan linker that connects the benzoic acid and pyrazolone fragments of the inhibitor is sandwiched between hydrophobic amino acid residues Leu873, Leu1014, and Pro1076. The pyrazolone fragment bearing phenyl and methyl groups in position 1 and 3, respectively, is located near Leu648, Phe649, Lys771, His875, Ser876, Val1011, and Phe1013. The oxygen atom of the pyrazolone carbonyl group forms a hydrogen bond with Ser876 (**Figure 4 (A)**).

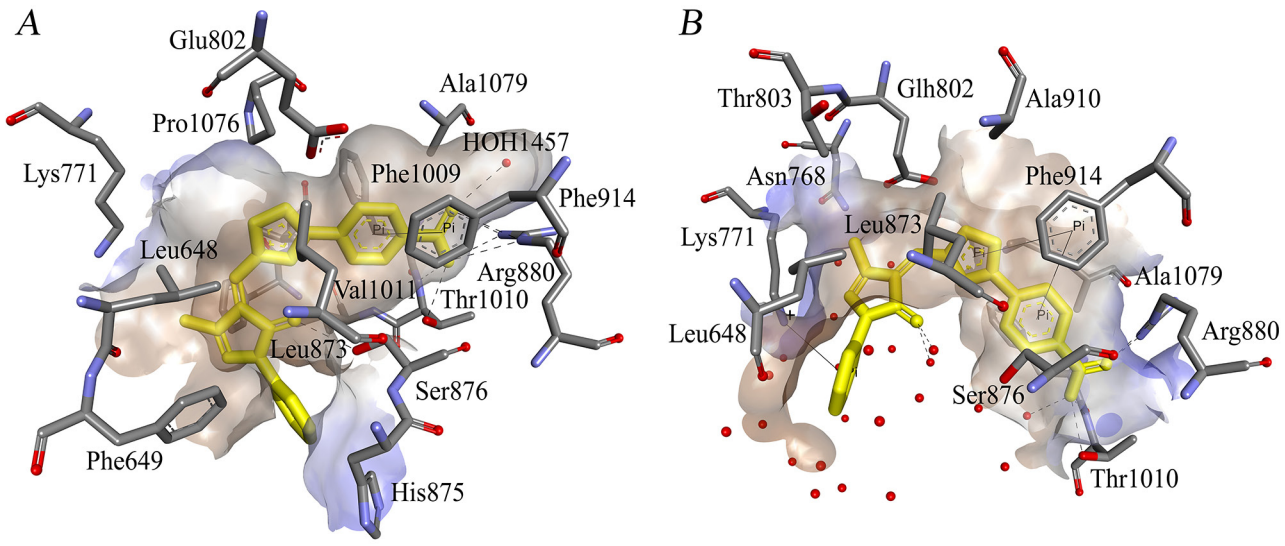


Figure 4. Binding modes of the *Z*-isomer of compound **3a** in the active site of XO predicted by the molecular docking calculation (*A*) and the subsequent molecular dynamic simulation (*B*). All the hydrogens are hidden for clarity, and the oxygen atoms shown as red spheres represent water molecules

ISSN 2308-8303 (Print) / 2518-1548 (Online)

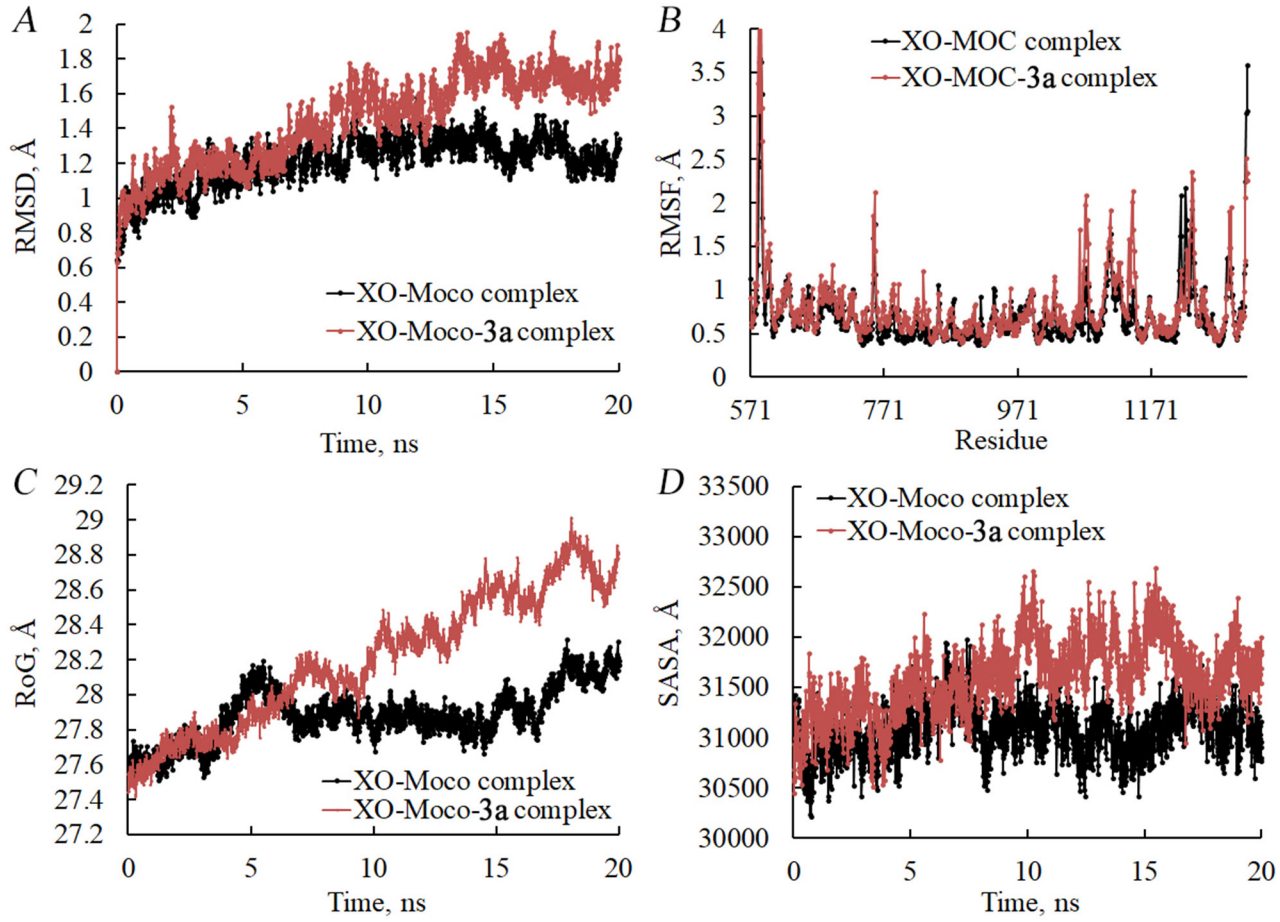


Figure 5. The values of the root mean square deviation of the enzyme backbone (*A*), the root mean square fluctuation of the amino acid residues (*B*), the radius of gyration (*C*), and the solvent accessible surface area (*D*) obtained from the MD simulation for the XO-Moco and XO-Moco-**3a** model systems

The molecular dynamic simulation was performed by the NAMD software [12] to verify the stability of the enzyme-inhibitor complex model obtained, as well as for a more detailed analysis of the interactions that could occur between inhibitor **3a** and the enzyme active site. The calculations were performed with the complex of the chain C of the enzyme containing molybdopterin and the inhibitor (XO-Moco-**3a**), as well as with the complex of the chain C of XO containing only a molybdopterin cofactor (XO-Moco). The time-dependent changes of the root mean square deviation (RMSD) values for the enzyme backbone atoms for both model systems are shown in **Figure 5 (A)**. As can be seen, the RMSD of XO-Moco and XO-Moco-**3a** complexes reached equilibration approximately after 10 ns and 14 ns, respectively. The XO-Moco-**3a** model system has higher RMSD values compared to those for the XO-Moco complex. This suggests that the binding of the ligand leads to a change in the structure of the enzyme. Despite this, the root mean square fluctuation (RMSF) revealed that compound **3a** only slightly affected the fluctuation of the nearby amino acid residues (**Figure 5 (B)**). The negative effect of the ligand on the compactness and folding of XO is indicated by the increased values of the radius of gyration (RoG) and solvent-accessible surface area (SASA) which are shown in **Figure 5 (C)** and **Figure 5 (D)**, respectively.

The binding mode of inhibitor **3a** in the active site of the XO obtained after 20 ns of the MD simulation is given in **Figure 4 (B)**. The ligand is more deeply located in the active site of the enzyme as compared with its location after the molecular docking (**Figure 4 (A)**). This is especially noticeable from the changed locations of the furan ring and the 3-methyl-5-phenyl substituted pyrazolone moiety, which showed π-π-stacking and π-cation interactions with Phe914 and Lys771, respectively. Such position of the inhibitor can be caused by the solvent action of water forming hydrogen bonds with the carbonyl oxygen of the pyrazolone ring. The changes in the structure of the enzyme detected by the plots in **Figure 5** can be related to the changes in the position of the inhibitor. According to several studies [13, 14, 15] and the check of the amino acid residues protonation states by the PROPKA software [16], Glu802 was protonated, and therefore, was represented as Glu802.

The free binding energy of compound **3a** in the active site of the XO was studied from the MD simulation results using the MMPBSA method. As seen from **Table 2**, the VDWAALS, EEL, and ENPOLAR energies contribute to stabilization of the enzyme-inhibitor complex, while the EPB and EDISPER energies lead to its destabilization.

The free energy decomposed by the MMPBSA method per amino acid residues located within 4 Å from the ligand in the enzyme active site at the last frame of the MD simulation are shown in **Table 3**. Currently, the method cannot be used for the decomposition of the non-polar solvation component. According to the total energies, the interactions of the benzoic acid moiety of the ligand with the amino acid residues Arg880, Phe1009, and Thr1010 are key factor for the enzyme-inhibitor complex stabilization. As can be seen, the electrostatic energies make the greatest contribution to these interactions.

Table 2. The calculated energies of MMPBSA components of the free binding energy of compound **3a** bound at the XO active site*

MMPBSA component	Energy ± SD, kcal mol^{-1}
VDWAALS	-34.5398 ± 3.1316
EEL	-101.2729 ± 14.6772
EPB	106.1432 ± 10.5794
ENPOLAR	-28.9013 ± 1.6140
EDISPER	49.3169 ± 1.9125
ΔG_{total}	-11.3089 ± 6.7691

Note: *VDWAALS is a van der Waals energy contribution from MM; EEL is the electrostatic energy as calculated by the MM force field; EPB is an electrostatic contribution to the solvation free energy calculated by PB; ENPOLAR is a nonpolar contribution to the solvation free energy calculated by an empirical model; EDISPER is dispersion energy; ΔG_{total} is the free binding energy.

Conclusions

Thus, pyrazolone-based 4-(furan-2-yl)benzoic acids **3a**–**i** can inhibit XO with IC_{50} values in the submicromolar range. The inhibitory properties of the compounds are affected by the nature of substituents in position 3 of the pyrazolone ring. The inhibition of XO by most of the compounds synthesized is significantly reduced in the presence of bovine serum albumin or Tween-80, excluding 3-methyl-1-phenyl pyrazolone-based derivative **3a**. Among the 4-(furan-2-yl)benzoic acids synthesized, compound **3a** has proven to be a potent inhibitor of xanthine oxidase. Kinetic studies have shown that the 3-methyl-1-phenyl pyrazolone-based 4-(furan-2-yl)benzoic acid **3a** is a mixed-type inhibitor of the enzyme. The inhibition constants calculated suggest that the affinity of the inhibitor to the free enzyme is higher than that to the enzyme-substrate complex. Molecular docking and molecular dynamic simulations have shown that the salt-bridge and the hydrogen bond formed between the carboxylic group of the inhibitor and the residues Arg880 and Thr1010 can be responsible for the stability of the enzyme-inhibitor complex.

Experimental part

Commercially available chemical reagents and solvents were purchased and used without purification. The TCL method was applied to monitor the reaction progress. The Fisher-Johns apparatus was used for the melting point determination. ^{1}H NMR spectra were taken on a Varian Mercury (500 MHz) spectrometer in DMSO-d_6 or $CF_3C(O)OD$ solution using the signal of residual

Table 3. The free energy decomposed per amino acid residues

Residue	Energy ± SD, kcal mol^{-1}			
	van der Waals	Electrostatic	Polar solvation	TOTAL
Leu648	-1.64 ± 0.57	-0.03 ± 0.30	0.65 ± 0.48	-1.02 ± 0.44
Asn768	-0.37 ± 0.15	-0.16 ± 0.27	0.88 ± 0.43	0.35 ± 0.44
Lys771	-1.05 ± 0.41	-15.21 ± 2.196	17.21 ± 2.97	0.94 ± 0.92
Glh802	-0.90 ± 0.33	-0.51 ± 0.24	1.78 ± 0.47	0.37 ± 0.46
Thr803	-0.21 ± 0.12	-0.18 ± 0.08	0.24 ± 0.11	-0.15 ± 0.11
Leu873	-1.83 ± 0.30	0.85 ± 0.17	-0.59 ± 0.16	-1.57 ± 0.33
Ser876	-1.37 ± 0.20	-0.12 ± 1.16	1.97 ± 1.10	0.47 ± 0.49
Arg880	0.13 ± 0.78	-39.28 ± 5.73	32.42 ± 2.62	-6.74 ± 3.77
Ala910	-0.18 ± 0.10	-0.57 ± 0.08	0.56 ± 0.11	-0.19 ± 0.15
Phe914	-1.30 ± 0.34	0.11 ± 0.24	0.23 ± 0.29	-0.96 ± 0.28
Phe1009	-2.03 ± 0.37	-5.35 ± 0.87	4.27 ± 0.35	-3.12 ± 0.94
Thr1010	0.43 ± 0.83	-12.82 ± 3.65	6.80 ± 1.68	-5.59 ± 1.92
Ala1078	-0.87 ± 0.24	-0.80 ± 0.37	1.00 ± 0.28	-0.68 ± 0.35
Ala1079	-1.09 ± 0.33	-0.38 ± 0.58	1.13 ± 0.49	-0.34 ± 0.46

ISSN 2308-8303 (Print) / 2518-1548 (Online)

solvent protons as a standard. IR spectra were recorded on a Vertex-70 spectrometer in KBr tablets. LC-MS spectra were obtained using an Agilent 1200 Series high-performance liquid chromatograph.

4-(5-Formyl-2-furanyl)benzoic acid **2** was obtained by the method described previously [17].

The general procedure for the synthesis of compounds 3a–i

A solution of 0.005 mol of corresponding pyrazolone **1** in 15–20 mL of acetic acid was added to a hot solution of 0.005 mol of 4-(5-formyl-2-furanyl)benzoic acid **2** in 20–30 mL of acetic acid followed by addition of 0.005 mol of ethanolamine. The reaction mixture was refluxed for 2 hours and then left at 20–25 °C for 12 hours. The precipitate formed was filtered, washed with ethanol, dried, and recrystallized from a mixture of MeCN/DMF.

4-[5-(3-Methyl-5-oxo-1-phenyl-1,5-dihydropyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3a**)

E/*Z* – 1:3. Burgundy solid. Yield – 86%. M. p. > 250 °C. Anal. Calcd for $C_{22}H_{16}N_2O_4$, %: C 70.96, H 4.33, N 7.52. Found, %: C 70.90, H 4.38, N 7.44. IR (KBr), ν_{max}, cm^{-1}: 2873, 1680 (C=O), 1604, 1319, 1273, 1027. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 2.31 (0.75H, s, Me), 2.68 (0.25H, s, Me), 7.18 (1H, t, J_{HH} = 8.0 Hz, Ar), 7.49–7.66 (2H, m, Ar), 7.75–8.00 (4H, m, Ar, –CH=), 8.00–8.05 (4H, m, Ar), 8.64 (1H, d, J_{HH} = 4.0 Hz, Ar), 13.03 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 373 $[M+H]^+$ (100).

4-[5-(5-Oxo-1-phenyl-3-trifluoromethyl-1,5-dihydro-pyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3b**)

Z-isomer. Burgundy solid. Yield – 82%. M. p. > 250 °C. Anal. Calcd for $C_{22}H_{13}F_3N_2O_4$, %: C 61.98, H 3.07, N 6.57. Found, %: C 62.04, H 3.16, N 6.50. IR (KBr), ν_{max}, cm^{-1}: 2535, 1692 (C=O), 1590, 1510, 1470, 1427, 1276, 1116, 954, 808. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.33 (1H, t, J_{HH} = 8.0 Hz, Ar), 7.50–7.53 (2H, m, Ar, –CH=), 7.68–7.72 (1H, m, Ar), 7.82–7.87 (3H, m, Ar), 8.07–8.17 (4H, m, Ar), 8.80–8.82 (1H, m, Ar), 13.09 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 427 $[M+H]^+$ (100).

4-[5-(5-Oxo-1,3-diphenyl-1,5-dihydro-pyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3c**)

E/*Z* – 1:5. Burgundy solid. Yield – 85%. M. p. > 250 °C. Anal. Calcd for $C_{27}H_{18}N_2O_4$, %: C 74.65, H 4.18, N 6.45. Found, %: C 74.74, H 4.22, N 6.40. IR (KBr), ν_{max}, cm^{-1}: 2545, 1696 (C=O), 1591, 1275, 942, 802. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.00 (1H, d, J_{HH} = 8.0 Hz, Ar), 7.25 (1H, t, J_{HH} = 8.0 Hz, Ar), 7.48 (2H, q, J_{HH} = 8.0 Hz, Ar), 7.61–7.79 (6H, m, Ar, –CH=), 7.78 (2H, d, J_{HH} = 8.0 Hz, Ar), 8.76–7.96 (5H, m, Ar), 12.17 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 436 $[M+H]^+$ (100).

4-{5-[3-(4-Fluoro-phenyl)-5-oxo-1-phenyl-1,5-dihydro-pyrazol-4-ylidenemethyl]-furan-2-yl}-benzoic acid (**3d**)

E/*Z* – 1:5. Burgundy solid. Yield – 85%. M. p. > 250 °C. Anal. Calcd for $C_{27}H_{17}FN_2O_4$, %: C 71.68, H 3.79, N 6.19. Found, %: C 71.72, H 3.82, N 6.12. IR (KBr), ν_{max}, cm^{-1}: 2989, 1696 (C=O), 1591, 1496, 1275, 944, 810. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.01 (1H, d, J_{HH} = 8.0 Hz, Ar), 7.22–7.32 (2H, m, Ar), 7.43–7.51 (4H, m, Ar), 7.64–7.86 (4H, m, Ar, –CH=), 7.95–8.76 (5H, m, Ar), 12.12 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 453 $[M+H]^+$ (100).

4-{5-[3-(4-Chloro-phenyl)-5-oxo-1-phenyl-1,5-dihydro-pyrazol-4-ylidenemethyl]-furan-2-yl}-benzoic acid (**3e**)

E/*Z* – 1:3. Burgundy solid. Yield – 80%. M. p. > 250 °C. Anal. Calcd for $C_{27}H_{17}ClN_2O_4$, %: C 69.16, H 3.65, Cl 7.56, N 5.97. Found, %: C 69.34, H 3.72, Cl 7.60, N 5.94. IR (KBr), ν_{max}, cm^{-1}: 2536, 1683 (C=O), 1592, 1274, 941, 803, 771. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 6.96–7.27 (1H, m, Ar), 7.46–7.52 (3H, m, Ar), 7.61–7.86 (6H, m, Ar, –CH=), 7.94–8.08 (5H, m, Ar), 8.75 (1H, d, J_{HH} = 4.0 Hz, Ar), 13.08 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 470 $[M+H]^+$ (100).

4-{5-[3-(4-Nitro-phenyl)-5-oxo-1-phenyl-1,5-dihydro-pyrazol-4-ylidenemethyl]-furan-2-yl}-benzoic acid (**3f**)

E/*Z* – 1:5. Burgundy solid. Yield – 80%. M. p. > 250 °C. Anal. Calcd for $C_{27}H_{17}N_3O_6$, %: C 67.64, H 3.57, N 8.76. Found, %: C 67.68, H 3.64, N 8.72. IR (KBr), ν_{max}, cm^{-1}: 2535, 1687 (C=O), 1592, 1516, 1348, 1273, 803. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 6.87-8.06 (6H, m, Ar, –CH=), 8.22–8.41 (2H, m, Ar), 8.74 (8H, m, Ar), 13.05 (br. s, 1H, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 480 $[M+H]^+$ (100).

4-[5-(5-Oxo-1-phenyl-3-thiophen-2-yl-1,5-dihydro-pyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3g**)

Z-izomer. Burgundy solid. Yield – 80%. M. p. > 250 °C. Anal. Calcd for $C_{25}H_{16}N_2O_4S$, %: C 68.17, H 3.66, N 6.36, S 7.28. Found, %: C 68.24, H 3.76, N 6.46, S 7.32. IR (KBr), ν_{max}, cm^{-1}: 2531, 1682 (C=O), 1593, 1422, 1277, 693. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 7.22 (2H, t, J_{HH} = 8.0 Hz, Ar), 7.30 (1H, t, J_{HH} = 4.0 Hz, Ar), 7.45 (2H, t, J_{HH} = 8.0 Hz, Ar), 7.58 (1H, d, J_{HH} = 4.0 Hz, Ar), 7.76–7.85 (3H, m, Ar, –CH=), 7.91–8.03 (5H, m, Ar), 8.72 (1H, d, J_{HH} = 4.0 Hz, Ar), 13.40 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 441 $[M+1]^+$ (100).

4-[5-(5-Oxo-1-phenyl-3-pyridin-4-yl-1,5-dihydro-pyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3h**)

E/*Z* – 1:5. Burgundy solid. Yield – 85%. M. p. > 250 °C. Anal. Calcd for $C_{26}H_{17}N_3O_4$, %: C 71.72, H 3.94, N 9.65. Found, %: C 71.86, H 4.00, N 9.60. IR (KBr), ν_{max}, cm^{-1}: 2920, 2567, 1670 (C=O), 1602, 1510, 1400, 1265, 931, 807. ^1H NMR (500 MHz, $CF_3C(O)OD$), δ, ppm: 7.42–7.80 (7H, m, Ar, –CH=), 8.21–8.75 (7H, m, Ar), 9.22–9.30 (2H, m, Ar). LC-MS, *m*/*z* (I_{rel}, %): 436 $[M+H]^+$ (100).

4-[5-(5-Oxo-3-phenyl-1-p-tolyl-1,5-dihydro-pyrazol-4-ylidenemethyl)-furan-2-yl]-benzoic acid (**3i**)

E/*Z* – 1:5. Burgundy solid. Yield – 87%. M. p. > 250 °C. Anal. Calcd for $C_{28}H_{20}N_2O_4$, %: C 74.99, H 4.50, N 6.25. Found, %: C 74.90, H 4.67, N 6.30. IR (KBr), ν_{max}, cm^{-1}: 2923, 2536, 1679 (C=O), 1603, 1513, 1424, 1276, 943, 812. ^1H NMR (500 MHz, DMSO-d_6), δ, ppm: 2.33 (3H, s, Me), 7.29 (2H, d, J_{HH} = 8.0 Hz, Ar), 7.60–7.64 (5H, m, Ar, –CH=), 7.78 (2H, d, J_{HH} = 8.0 Hz, Ar), 7.88 (2H, t, J_{HH} = 8.0 Hz, Ar), 8.05–8.77 (5H, m, Ar), 13.11 (1H, br. s, C(O)OH). LC-MS, *m*/*z* (I_{rel}, %): 449 $[M+H]^+$ (100).

The *in vitro* study of pyrazolone-containing 4-(furan-2-yl)benzoic acids as xanthine oxidase inhibitors

XO from bovine milk and xanthine as a substrate were purchased from Sigma-Aldrich. In the case of pyrazolone-based 4-(furan-2-yl)benzoic acids **3a**–**i**, the system contained a sodium-phosphate buffer (50 mM, pH 7.4), xanthine (50 μM), EDTA (0.1 mM), DMSO (1%), and an inhibitor. To study the specificity of the XO inhibition by compounds **3a**–**i**, the reaction mixture additionally contained 2 μM of BSA or 0.025 vol. % Tween-80. The reaction was initiated by the enzyme addition after preincubation of the mixture for 5 min. The total volume of the system was 2 mL. The activity of XO was monitored spectrophotometrically at 293 nm. The uric acid molar extinction coefficient of 12.2 mM^{-1} cm^{-1} was used for calculations. The value of the calculated Michaelis-Menten constant (K_m) was 5.7 μM.

The fluorescence quenching experiment

The fluorescence quenching studies were performed on the example of compound **3i** to confirm the interaction of pyrazolone-based 4-(furan-2-yl) benzoic acids with bovine serum albumin (BSA). The reaction mixture consisted of sodium phosphate buffer (50 mM, pH 7.4), 2 μM BSA, DMSO (1%), and a quencher (compound **3i**). The total volume of the reaction mixture was 2 mL. The Stern-Volmer quenching constants were obtained from plots of F_0/F *vs.* the quencher concentration described by the Stern-Volmer equation:

$$F_0 / F = 1 + k_q\tau_0 = 1 + K_{SV}[Q]$$

where F_0 and F are the BSA fluorescence intensities observed without and with the quencher in the model systems, respectively; [*Q*] is the concentration of the quencher; k_q is the apparent bimolecular quenching rate constant; τ_0 is the lifetime of unquenched tryptophan in BSA; K_{SV} is the Stern-Volmer quenching constant.

Molecular docking calculation

Compound **3a** was docked into the active site (chain C) of XO from bovine milk (PDB code 1FIQ [9]) using the protocol described in [18]. The calculation was performed by the AutoDock Vina software [11]. The enzyme-inhibitor model complex was analyzed by Discovery Studio 3.5 (Accelrys, San Diego, CA, USA).

Molecular dynamic simulation

The molecular dynamic simulations were performed using the NAMD software [12] according to the previously described protocol [19]. The preparation of the model system for the calculation was carried out using the conda environment. The VMD 1.9.3 [20] was used for the calculation of RMSD, RMSF, RoG, and SASA. The binding free energy and its decomposition per amino acid residues were performed by MMPBSA.py [21] according to the protocol described in [19].

Acknowledgements

We would like to thank Enamine Ltd for supporting the synthesis of compounds.

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Journal of Organic and Pharmaceutical Chemistry

Original Research

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UDC 542.913+547.512+547.513

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The Synthesis of Functionalized Dimethylphosphinoyl Cyclopropanes and Cyclobutanes

Abstract

A simple preparative approach to a series of functionalized dimethylphosphinoyl-containing cyclopropanes and cyclobutanes has been developed; it is based on cyclocondensation of dimethylphosphinoyl acetonitrile with 1,2- and 1,3-dibromoalkanes. Synthetic procedures for obtaining nitriles, amines and carboxylic acids containing in their structure small saturated cyclic rings of cyclopropane or cyclobutane and a dimethylphosphine oxide fragment, which are popular in drug design, have been developed.

Keywords: dimethylphosphine oxides; cyclopropane; cyclobutane; amines; carboxylic acids

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Синтез функціоналізованих диметилфосфіноїл циклопропанів і циклобутанів

Анотація

Розроблено зручний препаративний підхід до ряду функціоналізованих диметилфосфіноїлвмісних циклопропанів і циклобутанів, основою якого є циклоконденсація диметилфосфіноїлацетонітрилу з 1,2- та 1,3-дибромоалканами. Розроблено синтетичні процедури для одержання нітрилів, амінів і карбонових кислот, які поєднують у своїй структурі популярні в drug-дизайні малі насичені циклічні системи циклопропану або циклобутану і диметилфосфіноксидний фрагмент.

Ключові слова: диметилфосфіноксиди; циклопропан; циклобутан; аміни; карбонові кислоти

Citation: Aleksandrova, A. M.; Cherednichenko, A. S.; Rassukana, Yu. V. The Synthesis of Functionalized Dimethylphosphinoyl Cyclopropanes and Cyclobutanes. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 36–42.
https://doi.org/10.24959/ophcj.23.299237
Received: *4 November 2023*; **Revised:** *22 November 2023*; **Accepted:** *26 November 2023*

Funding: The author received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

The introduction of small saturated rigid carbocycles, specifically, cyclopropane (*Cy*Pr) and cyclobutane (*Cy*Bu), into the structure of biologically active compounds is an effective tool for improving their physicochemical and ADME (absorption, distribution, metabolism, excretion) properties [1]. The cyclopropyl group is present in more than 40 FDA-approved drugs used in the treatment of cancer, viral and bacterial infections, diabetes, CNS disorders, cardiovascular and musculoskeletal system diseases, etc. (**Figure**, compounds **A**–**F**) [2]. The unique properties of the cyclopropane ring (coplanarity of carbon atoms, increased *p*-character of the C-C bonds and increased energy of the C-H bonds, high ring strain) enhance the resistance of molecules to metabolic degradation *in vivo*, lead to a decrease in their lipophilicity and conformational lability, and allow to apply cyclopropyl as an isostere of an olefin bond, as well as *i*Pr, Ph, and *tert*-Bu groups [3–7].

In turn, replacing the aliphatic part of the molecule with 1,2- or 1,3-disubstituted cyclobutane

ISSN 2308-8303 (Print) / 2518-1548 (Online)

ring allows to increase their conformational rigidity, prevents an undesirable *cis*/*trans*-isomerization and, as a result, leads to improved binding with the biological target (**Figure**, compounds **G**–**H**). Additionally, cyclobutane can act as a bioisoster of *gem*-dimethyl and aryl groups [1, 8]. Thus, the development of synthetic approaches to compounds that contain a mono-/polysubstituted cyclopropyl or cyclobutyl nucleus is definitely an important task of medicinal chemistry.

On the other hand, after the approval of the anticancer drug brigatinib (**Figure**, compound **I**) in 2017 [9, 10], which bears a dimethylphosphine oxide function, there is a growing interest in compounds modified by the $Me_2P(O)$ moiety. Unlike phosphates and phosphonates, which are more usual in drug design [11], phosphine oxides represent a new and promising chapter of phosphorus-containing pharmaceuticals. It is due to the nature of the dimethylphosphine oxide group, which provides better metabolic stability and bioavailability of the respective compounds as, unlike phosphoric acid derivatives, it does not undergo ionization at physiological pH. Moreover, dimethylphosphine oxides, usually, demonstrate high water solubility and the ability to form hydrogen bonds, which facilitates the transportation of the substances in the body, as well as their interaction with a target protein [12]. Taking into account that modern criteria for the design of pharmaceuticals favor molecules with an increased level of saturation (Fsp^3), [13–15] the development of new methods for obtaining saturated aliphatic dimethylphosphine oxides is an urgent task.

However, only a few examples of the synthesis of saturated building blocks with the $Me_2P(O)$ function are currently known, and the vast majority of them have a heterocyclic (oxirane, azetidine, pyrrolidine, or piperidine) core [16–18]. Moreover, despite the high relevance of the presence of a cyclopropyl or cyclobutyl fragment

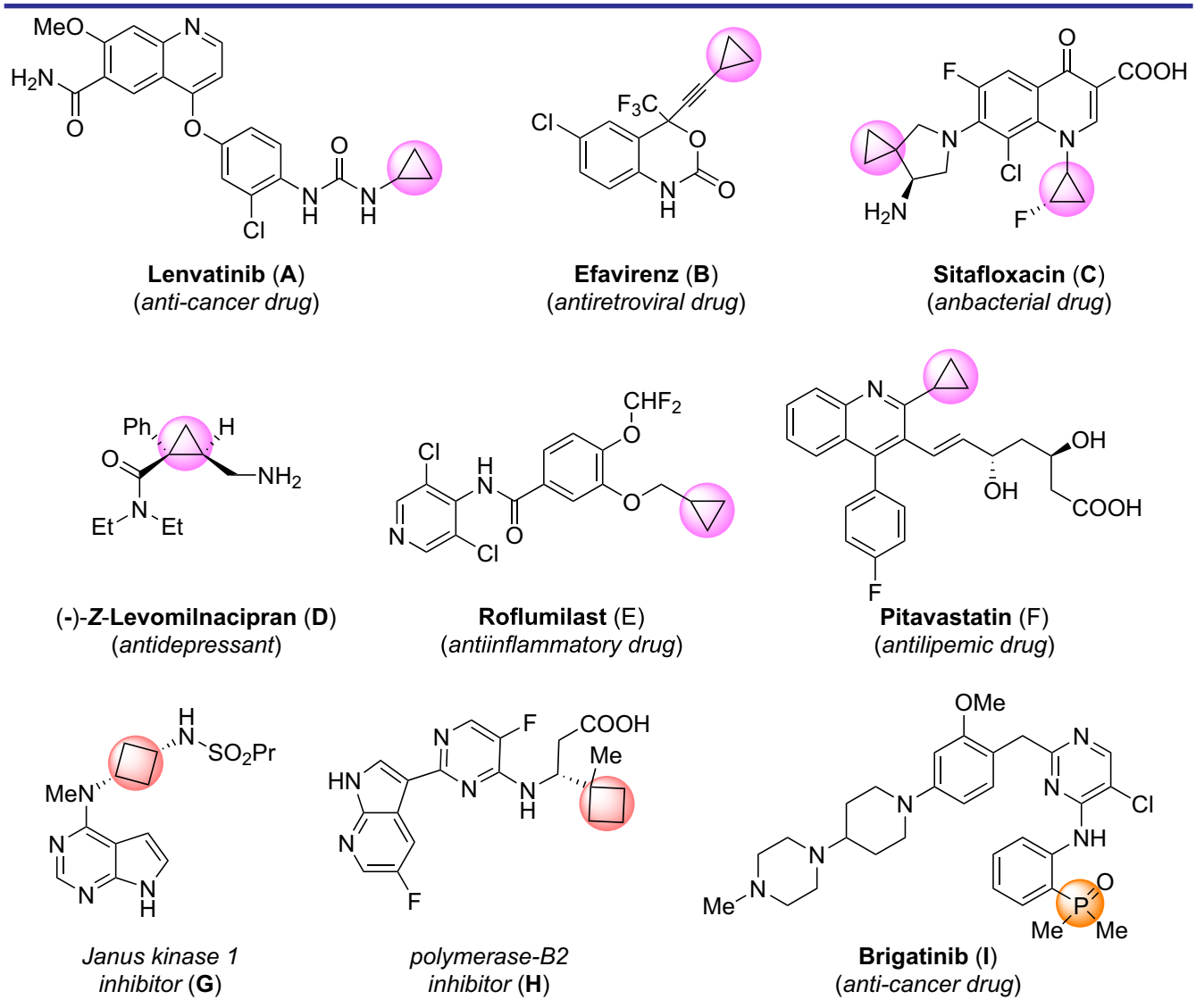


Figure. Approved drugs and drug candidates that contain a cyclopropyl-, cyclobutyl- or dimethylphosphine oxide moiety

in a molecule for drug discovery, any synthetic approaches to dimethylphosphinoyl derivatives of cyclopropane or cyclobutane were unknown. Therefore, this work aims to fill this gap and is devoted to the synthesis of dimethylphosphinoyl-containing cyclopropanes and cyclobutanes, bearing functional groups that can be used as building blocks for synthetic and medicinal chemistry.

■ Results and discussion

Previously, we developed a preparative method for the synthesis of dimethylphosphinoyl acetonitrile (**1**) based on the interaction of chloroacetonitrile with (trimethylsilyl)dimethylphosphinite generated *in situ* from dimethylphosphine oxide and trimethylsilyl chloride in the presence of the Hunig's base (**Scheme 1**) [16]. This approach allows obtaining phosphine oxide derived acetonitrile **1** with a high yield (65%) in multigram quantities (>50 g).

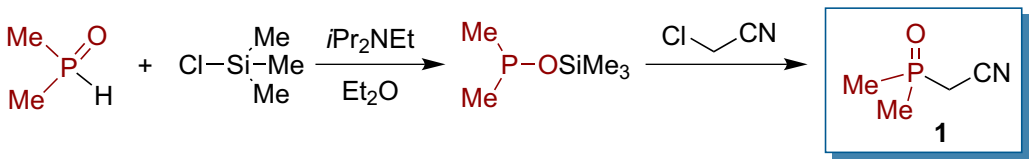
Dimethylphosphinoyl acetonitrile (**1**) contains a methylene component activated by two strong electron-withdrawing groups, which alkylation with 1,2- and 1,3-dibromoalkanes allows the formation of cyclopropyl and cyclobutyl rings, respectively. Indeed, the cyclocondensation of nitrile **1** with 1,2-dibromoethane and 1,3-dibromopropane occurs chemoselectively in the presence of sodium hydride (THF, 65 °C) leading to the formation of C-phosphinoyl-containing nitriles of cyclopropanoic (**2**) or cyclobutanoic (**3**) acids (**Scheme 2**).

The high aqueous solubility of dimethylphosphine oxides is a greatly desired feature for improved drug bioavailability, but it frequently complicates isolation of these compounds and leads to a significant decrease in reaction yields. Considering this we could not use the standard procedure, when isolating compounds **2** and **3**, and remove inorganic impurities from the reaction mixture by treating it with water. Instead, the inorganic precipitate was carefully (in order to prevent ignition of the remaining sodium hydride) filtered off, and the resulting solution was evaporated. The crude residue was recrystallized from MTBE to give pure nitriles **2** and **3** as low-melting hygroscopic crystals that could be stored for a long period in an anhydrous atmosphere.

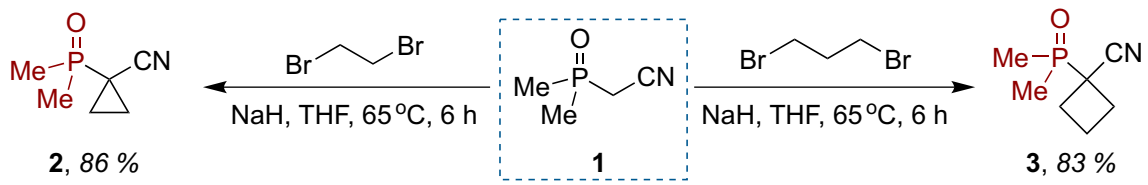
An interesting spectral characteristic of dimethylphosphinoyl cyanocyclopropane **2** is the shift of the doublet of the quaternary carbon atom in ^{13}C NMR spectrum, which is observed around 6.7 ppm. ($^{1}J_{CP}$ = 67 Hz) compared to its *Cy*Bu-substituted analog **3**, for which the signal is located at 33.57 ppm. It is consistent with the previous data, which indicate a shift of the signals of cyclopropane ring carbon atoms, especially those bearing shielding substituents (CN, COOH), to a stronger field, due to the special nature of C-C bonds of the cyclopropane ring [19, 20].

The presence of the C≡N group in cyclopropyl and cyclobutylphosphine oxides **2** and **3** allows using them as substrates for the synthesis of various classes of organic compounds, such as carboxylic acids, amides, amines, etc. Therefore, the next step of our study was to develop methods of reduction and hydrolysis of the nitrile function of nitriles **2**, **3** in order to obtain valuable dimethylphosphinoyl-substituted amines and acids as potential building blocks for drug discovery.

Thus, the reduction of the nitrile group of dimethylphosphine oxides **2** and **3** to the aminomethyl function was easily achieved by Ni-catalyzed hydrogenation in EtOH/NH_3 solution resulting in the formation of dimethylphosphinoyl-substituted ß-aminomethyl cyclopropane **4** and cyclobutane **5**

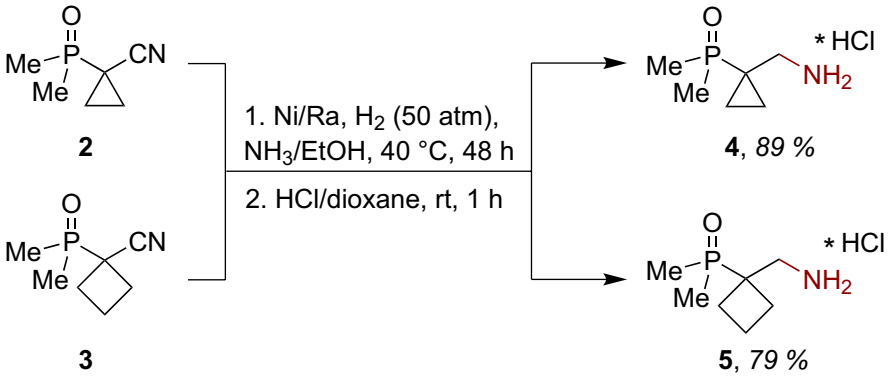


Scheme 1. The synthetic approach to dimethylphosphinoylacetonitrile reported previously [16]



Scheme 2. Dimethylphosphinoyl acetonitrile in the synthesis of dimethylphosphinoyl-substituted nitriles of cyclopropanoic and cyclobutanoic acids

ISSN 2308-8303 (Print) / 2518-1548 (Online)



Scheme 3. Reduction of the nitrile function of dimethylphosphinoyl cyanocyclopropane and cyclobutane

isolated as hydrochlorides with yields of 89% and 79%, respectively (**Scheme 3**).

The hydrolysis of the nitrile group of (*Cy*Pr)dimethylphosphine oxide **2** upon heating the substrate in the concentrated hydrochloric acid (90 °C, 6 hrs) was accompanied by the partial destruction of the cyclopropane cycle, and as a result, a complex mixture was formed. Instead, the hydrolysis of **2** with potassium hydroxide followed by careful acidification with an HCl/dioxane solution occurred selectively, and it allowed obtaining the target cyclopropylcarboxylic acid **6** isolated in an analytically pure state with the yield of 65% (**Scheme 4**).

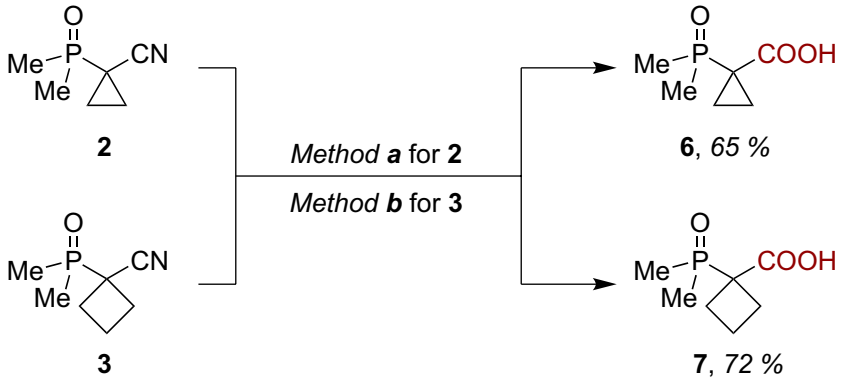
In the case of (*Cy*Bu)dimethylphosphine oxide **3**, the hydrolysis with the concentrated hydrochloric acid proceeds chemoselectively and leads to a water-soluble cyclobutanecarboxylic acid **7**, isolated with the yield of 72%.

At the same time, dimethylphosphinoyl derivatives of cyclopropanoic and cyclobutanoic acids **6** and **7** can be involved in the Curtius reaction, which opens the way to geminal aminophosphine oxides. It has been found that the best results can be achieved when using diphenylphosphoryl azide (DPPA) in the presence of *tert*-butanol. Thus, acids **6** and **7** react with DPPA in benzene or toluene at 90°C, the following treatment of the reaction mixture with *tert*-butyl alcohol leads to the formation of *N*-Boc aminophosphine oxides **8** and **9**. The following removal of the *tert*-butoxycarbonyl group was successfully achieved in mild conditions by the treatment with a HCl/dioxane solution, as a result the corresponding amines **10** and **11** were obtained as hydrochlorides with high yields (**Scheme 5**).

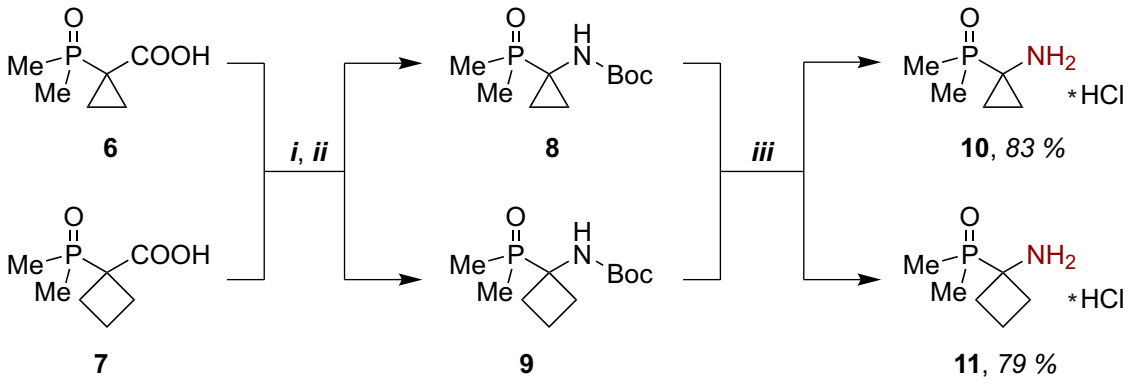
α- and ß-Aminophosphine oxides **4**, **5** and **10**, **11** are promising substrates for the synthesis of biologically active compounds as they will allow the targeted introduction of both a dimethylphosphine oxide moiety and a saturated cyclopropane or cyclobutane framework into the structure of a molecule.

■ Conclusions

A preparative method for the synthesis of synthetically attractive dimethylphosphinoyl-substituted nitriles of cyclopropanoic and cyclobutanoic acids has been developed. Their potential in obtaining small saturated cyclic amines and carboxylic acids with a dimethylphosphine oxide



Scheme 4. The synthetic approach to dimethylphosphinoyl-containing cyclopropanoic and cyclobutanoic acids



i: DPPA, Et_3N, toluene, 90 °C, 2 h; ***ii***: *t*BuOH, 90 °C, 3 h; ***iii***: HCl/dioxane, rt, 2 h

Scheme 5. Dimethylphosphinoyl derivatives of cyclopropanoic and cyclobutanoic acids in the Curtius reaction

group that are interesting building blocks for medical chemistry and drug development has been shown.

■ Experimental part

NMR spectra were recorded on a Bruker Avance DRX 600 spectrometer (operating frequency 150.8 MHz for ^{13}C); a Bruker Avance DRX 500 spectrometer (operating frequencies 499.9 MHz for ^{1}H, 125.7 MHz for ^{13}C, 161.9 MHz for ^{31}P); a Varian Unity Plus 400 instrument (operating frequencies 399.9 MHz for ^{1}H and 100.6 MHz for ^{13}C); a Mercury Varian Unity Plus 300 instrument (operating frequencies 301.5 MHz for ^{1}H and 75.8 MHz for ^{13}C). Chemical shifts were reported relative to internal TMS (^{1}H, ^{13}C), and H_3PO_4 (^{31}P) standards. Solvents were dried according to the standard procedures. Starting materials were purchased from Merck, Fluka, and Enamine Ldt. Melting points were uncorrected. The elemental analysis was carried out in the analytical laboratory of the Institute of Organic Chemistry, NAS of Ukraine.

The general procedure for the synthesis of compounds 2, 3

Nitrile **1** (10.0 g, 85.4 mmol) was added portionwise to a suspension of NaH (5.1 g, 213.5 mmol) in THF (100 mL) at 0–5 °C. The mixture was stirred at 20 °C for 1 h. Then the corresponding dibromoalkane (85.4 mmol) was added in one portion, and the mixture was refluxed for 5 h. After that it was cooled down to room temperature, the precipitate was filtered off, washed with CH_2Cl_2 (2×100 mL), the filtrate was concentrated *in vacuo*. The residue was recrystallized from MTBE (50 mL).

(1-Dimethylphosphinoyl)cyclopropane-1-carbonitrile (**2**)

A white crystalline solid. Yield – 10.5 g (86%). M. p. 76–78 °C. Anal. Calcd for $C_6H_{10}NOP$, %: C 50.35, H 7.04, N 9.79. Found, %: C 50.28, H 7.09, N 9.89. ^{1}H NMR (399.9 MHz, $CDCl_3$), δ, ppm: 1.40–1.46 (2H, m, CH_2), 1.48–1.55 (2H, m, CH_2), 1.67 (6H, d, $^{2}J_{HP}$ = 12.9 Hz, CH_3P). ^{13}C NMR (125.7 MHz, $CDCl_3$), δ, ppm: 6.70 (d, $^{1}J_{CP}$ = 67 Hz, $\underline{C}CN$), 11.57 (d, $^{2}J_{CP}$ = 8 Hz, CH_2), 14.84 (d, $^{1}J_{CP}$ = 70 Hz, CH_3P), 119.19 (d, $^{2}J_{CP}$ = 4 Hz, $C\underline{C}N$). ^{31}P NMR (161.9 MHz, $CDCl_3$), δ, ppm: 39.3.

(1-Dimethylphosphinoyl)cyclobutane-1-carbonitrile (**3**)

A yellowish crystalline solid. Yield – 11.2 g (83%). M. p. 67–68 °C. Anal. Calcd. for $C_7H_{12}NOP$, %: C 53.50, H 7.70, N 8.91. Found, %: C 53.35, H 7.79, N 8.99. ^{1}H NMR (399.9 MHz, $CDCl_3$), δ, ppm: 1.53 (6H, d, $^{2}J_{HP}$ = 12.5 Hz, CH_3P), 2.09–2.20 (1H, m, CH_2), 2.26–2.37 (1H, m, CH_2), 2.42–2.51 (2H, m, CH_2), 2.72–2.83 (2H, m, CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$), δ, ppm: 12.08 (d, $^{1}J_{CP}$ = 70 Hz, CH_3P), 17.56 (d, $^{3}J_{CP}$ = 8 Hz, CH_2), 25.87 (d, $^{2}J_{CP}$ = 4 Hz, CH_2), 33.57 (d, $^{1}J_{CP}$ 67 = Hz, $\underline{C}CN$), 120.80 (d, $^{2}J_{CP}$ = 4 Hz, $C\underline{C}N$). ^{31}P NMR (161.9 MHz, $CDCl_3$), δ, ppm: 42.9.

The general procedure for the synthesis of compounds 4, 5

A mixture of nitrile **2** or **3** (52.7 mmol) and Ni/Ra (0.5 g) in NH_3/EtOH (25 mL) was hydrogenated at 50 atm. and 40 °C for 48 h. The catalyst was removed by filtration, the filtrate was concentrated *in vacuo*, and the residue was dissolved in MTBE (100 mL) and triturated with HCl/dioxane solution (20 mL). The precipitate was collected by filtration, washed with acetone (20 mL) and dried *in vacuo*.

(1-(Aminomethyl)cyclopropyl)dimethylphosphine oxide hydrochloride (**4**)

Compound **4** was obtained from nitrile **2** (7.5 g, 52.7 mmol) as a white crystalline solid with the yield of 89% (8.6 g).

M. p. 234–235 °C. Anal. Calcd. for $C_6H_{15}ClNOP$, %: C 39.25, H 8.23, Cl 19.31, N 7.63.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

Found, %: C 39.37, H 8.16, Cl 19.22, N 7.67. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 0.94–1.02 (4H, m, CH_2), 1.46 (6H, d, $^{2}J_{HP}$ = 13.0 Hz, CH_3P), 2.99–3.08 (2H, m, CH_2), 8.15 (3H, br. s, NH_2*HCl). ^{13}C NMR (75.8 MHz, DMSO-d_6), δ, ppm: 9.62 (s, CH_2), 13.86 (d, $^{1}J_{CP}$ = 70 Hz, CH_3P), 15.82 (d, $^{1}J_{CP}$ = 94 Hz, CP(O)Me_2), 42.58 (d, $^{2}J_{CP}$ = 5 Hz, CH_2NH_2). ^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 50.6.

(1-(Aminomethyl)cyclobutyl)dimethylphosphine oxide hydrochloride (**5**)

Compound **5** was obtained from nitrile **3** (8.3 g, 52.7 mmol) as a white crystalline solid with the yield of 79% (8.2 g).

M. p. 215–218 °C. Anal. Calcd. for $C_7H_{17}ClNOP$, %: C 42.54, H 8.67, Cl 17.94, N 7.09. Found, %: C 42.47, H 8.61, Cl 17.92, N 7.17. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 1.53 (6H, d, $^{2}J_{HP}$ = 13.1 Hz, CH_3P), 1.79–1.90 (1H, m, CH_2), 1.99–2.32 (5H, m, CH_2), 3.27 (2H, d, $^{3}J_{HP}$ = 13.1 Hz, CH_2), 8.18 (3H, br. s, NH_2*HCl). ^{13}C NMR (100.6 MHz, D_2O), δ, ppm: 10.58 (d, $^{1}J_{CP}$ = 67 Hz, CH_3P), 15.03 (d, J_{CP} = 8 Hz, CH_2), 23.90 (d, J_{CP} = 2 Hz, CH_2), 37.62 (d, $^{1}J_{CP}$ = 67 Hz, CP(O)Me_2), 43.38 (d, $^{2}J_{CP}$ = 5 Hz, CH_2NH_2).^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 54.9.

(1-Dimethylphosphinoyl)cyclopropane-1-carboxylic acid (6)

Nitrile **2** (10 g, 69.9 mmol) was added to a solution of KOH (3.3 g, 139.7 mmol) in H_2O (50 mL), and the mixture was heated at 90 °C for 16 h. Then it was concentrated *in vacuo*, and HCl/dioxane (10 mL) was added to the residue. The suspension was triturated with ethanol (20 mL), and the precipitate was filtered off. The filtrate was evaporated, and the residue was triturated with acetone (2×20 mL), the precipitate was collected by filtration to give **6** as a white crystalline solid.

Yield – 7.4 g (65%). M. p. >170 °C (decomp.). Anal. Calcd. for $C_6H_{11}O_3P$, %: C 44.45, H 6.84. Found, %: C 44.56, H 6.81. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 1.24–1.26 (4H, m, CH_2), 1.55 (6H, d, $^{2}J_{HP}$ = 13.6 Hz, CH_3P), 12.89 (1H, br. s, COOH). ^{13}C NMR (75.8 MHz, DMSO-d_6), δ, ppm: 12.52 (s, CH_2), 15.67 (d, $^{1}J_{CP}$ = 74 Hz, CH_3P), 28.49 (br. s, $\underline{C}$COOH), 172.52 (s, $\underline{C}$OOH). ^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 39.3.

(1-Dimethylphosphinoyl)cyclobutane-1-carboxylic acid (7)

A solution of nitrile **3** (10 g, 63.6 mmol) in conc. HCl aqueous solution (50 mL) was heated at 90 °C for 16 h. After that the mixture was cooled down to room temperature, it was concentrated to dryness *in vacuo* and triturated with acetone (100 mL). The precipitate was filtered off, the filtrate was concentrated *in vacuo* to dryness. The residue was triturated with MeCN (50 mL), and the precipitate was collected by filtration to give acid **7** as a white crystalline solid.

Yield – 8.1 g (72%). M. p. 234–235 °C. Anal. Calcd. for $C_7H_{13}O_3P$, %: C 47.73, H 7.44. Found, %: C 47.87, H 7.42. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 1.39 (6H, d, $^{2}J_{HP}$ = 13.0 Hz, CH_3P), 1.84–1.95 (2H, m, CH_2), 2.39–2.50 (4H, m, CH_2), 12.82 (1H, br. s, COOH). ^{13}C NMR (125.7 MHz, DMSO-d_6)б δ, ppm: 12.65 (d, $^{1}J_{CP}$ = 68 Hz, CH_3P), 16.19 (d, $^{3}J_{CP}$ = 7 Hz, CH_2), 25.70 (d, $^{2}J_{CP}$ = 4 Hz, CH_2), 48.25 (d, $^{1}J_{CP}$ = 58 Hz, $\underline{C}$COOH), 174.48 (d, $^{2}J_{CP}$ = 3 Hz, $\underline{C}$OOH). ^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 43.9.

The general procedure for the synthesis of compounds 10 and 11

Diphenylphosphoryl azide (10 g, 36.7 mmol, 7.8 mL) was added to a solution of the corresponding acid **6**/**7** (34.9 mmol) and Et_3N (3.9 g, 38.5 mmol, 5.4 mL) in toluene (200 mL) at 90 °C. The resulting mixture was heated at this temperature until the gas release stopped, then *tert*-butanol (13 g, 175.0 mmol, 16 mL) was added in one portion, and the mixture was heated for additional 3 h. The solvent was evaporated, dioxane (50 mL) was added to the residue, followed by KOH (7 g, 175.0 mmol), and the suspension was stirred at room temperature for 2 h. The precipitate was filtered off, the filtrate was triturated with HCl/dioxane (10 mL) for 2 hrs. The precipitate was collected by filtration, washed with acetone (2×50 mL) and dried *in vacuo*.

(1-Aminocyclopropyl)dimethylphosphine oxide hydrochloride (**10**)

Compound **10** was obtained from acid **6** (5.0 g, 34.9 mmol) as a white crystalline solid.

Yield – 4.7 g (79%). M. p. >198 °C (decomp.). Anal. Calcd. for $C_6H_{15}ClNOP$, %: C 35.41, H 7.73, Cl 20.91, N 8.26. Found, %: C 35.57, H 7.78, Cl 20.83, N 8.31. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 1.10–1.19 (2H, m, CH_2), 1.38 (2H, d, $^{3}J_{HP}$ = 12.9 Hz, CH_2), 1.59 (6H, d, $^{2}J_{HP}$ = 13.3 Hz, CH_3P), 8.97 (3H, br. s, NH_2*HCl). ^{13}C NMR (75.8 MHz, D_2O), δ, ppm: 9.61 (s, CH_2), 13.33 (d, $^{1}J_{CP}$ = 72 Hz, CH_3P), 30.18 (d, $^{1}J_{CP}$ = 104 Hz, CP(O)Me_2). ^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 40.1.

(1-Aminocyclobutyl)dimethylphosphine oxide hydrochloride (**11**)

Compound **11** was obtained from acid **7** (5.0 g, 34.9 mmol) as a white crystalline solid.

Yield – 5.3 g (83%). M. p. >193 °C (decomp.). Anal. Calcd. for $C_6H_{15}ClNOP$, %: C 39.25, H 8.23, Cl 19.31, N 7.63. Found, %: C 39.39, H 8.28, Cl

19.33, N 7.61. ^{1}H NMR (399.9 MHz, DMSO-d_6), δ, ppm: 1.59 (6H, d, $^{2}J_{HP}$ = 13.1 Hz, CH_3P), 1.73–1.87 (1H, m, CH_2), 2.00–2.10 (1H, m, CH_2), 2.34–2.49 (4H, m, CH_2), 9.23 (3H, d, $^{3}J_{HP}$ = 7.1 Hz, NH_2*HCl). ^{13}C NMR (75.8 MHz, DMSO-d_6), δ, ppm: 12.08 (d, $^{1}J_{CP}$ = 68 Hz, CH_3P), 14.35 (s, CH_2), 26.55 (s, CH_2), 53.16 (d, $^{1}J_{CP}$ = 70 Hz, CNH_2). ^{31}P NMR (161.9 MHz, DMSO-d_6), δ, ppm: 45.5.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)

Journal of Organic and Pharmaceutical Chemistry

Advanced Research

OPEN ACCESS
Freely available online on
http://ophcj.nuph.edu.ua

UDC 54.057:547-316

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Nature-Inspired Tetrahydropentalene Building Blocks: Scalable Synthesis for Medicinal Chemistry Needs

Abstract

Inspired by the bioactivity of natural compounds with a bicyclo[3.3.0]octane core, the study focuses on developing tetrahydropentalene-2,5-dione (2,5-THP-dione) derivatives as potential building blocks for the use in medicinal chemistry. Using the commercially available 2,5-THP-dione, a number of alkylated derivatives and a monofunctional ketone were synthesized. Using optimized protocols for synthesis, target compounds were obtained with high yields on a multigram scale. These compounds are promising derivatives for further chemical derivatization and therapeutic use, and thus highlight the value of 2,5-THP-dione in creating complex molecular structures for drug discovery, as well as the importance of tetrahydropentalene derivatives as valuable building blocks in synthetic chemistry.

Keywords: tetrahydropentalene; building blocks; medicinal chemistry; multigram synthesis

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Білдинг-блоки на основі тетрагідропенталену, інспіровані природою: масштабований синтез для потреб медичної хімії

Анотація

Пропоноване дослідження, інспіроване біоактивністю природних сполук із біцикло[3.3.0]октановим ядром, зосереджено на розробці похідних тетрагідропентален-2,5-діону (2,5-THP-діону) як потенційних білдинг-блоків для застосування в медичній хімії. Використовуючи комерційно доступний 2,5-THP-діон, синтезували ряд його алкілованих похідних, а також монокето-похідну. Використовуючи оптимізовані протоколи синтезу, одержали цільові сполуки з високими виходами у мультиграмових кількостях. Синтезовані сполуки є перспективні похідні для подальшої хімічної дериватизації та терапевтичного застосування, що засвідчує цінність 2,5-THP-діону для створення складних молекулярних структур у процесі розроблення ліків, а також важливість похідних тетрагідропенталену як білдинг-блоків у синтетичній хімії.

Ключові слова: тетрагідропентален; білдинг-блоки; медична хімія; багатограмовий синтез

Citation: Smyrnov, O. K.; Pashenko, O. Ye. Nature-Inspired Tetrahydropentalene Building Blocks: Scalable Synthesis for Medicinal Chemistry Needs. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 43–50.
https://doi.org/10.24959/ophcj.23.299612
Supporting information: Copies of ^{1}H, ^{13}C (1D and 2D) NMR spectra of the synthesized compounds.
Received: *28 September 2023*; **Revised:** *1 November 2023*; **Accepted:** *4 November 2023*

Funding: The authors received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

Introduction

The quest to harness the potential of tetrahydropentalene (THP) derivatives in medicinal chemistry is driven by the remarkable biological activities observed for natural products featuring the bicyclo[3.3.0]octane core, such as carbacyclin (**1, Figure**), and clinprost (**3**). These compounds show prostaglandin analogs and platelet aggregation inhibitory properties, along with others displaying the anticancer activity like cylindramide (**2**), and antibiotic potential like geodin A (**4**). In general, they exemplify the diverse biological actions – from antimicrobial to enzyme-inhibiting activities – that THP structural motif offers [1]. The synthetic analogs of tetrahydropentalenes, thus, emerge as formidable scaffolds in the drug development, opening the opportunities for finding new therapeutic agents capable of targeting a broad spectrum of diseases.

The known synthetic biologically active bicyclo[3.3.0]octane derivatives were found to be potent dipeptidyl peptidase 4 (DPP-4) inhibitors for managing type 2 diabetes [2]. Meanwhile, the research of *Mitcheltree et al.* into bicyclic inhibitors of human arginase for cancer immunotherapy further exemplifies the potential of these derivatives in combatting severe health conditions [3].

In the realm of medicinal and synthetic chemistry, tetrahydropentalene derivatives stand out not only for their significant biological activity, but also for their versatility as intermediates. The synthetic routes towards biologically active tetrahydropentalene derivatives employ modern organic chemistry methods in search of potent THP-based compounds. The development of functionalized pentalenes *via* carbonyl-ene reactions and enzymatic kinetic resolution exemplifies the creative approaches undertaken to access these elusive compounds [4]. Additionally, the synthesis of racemic 1-desoxyhypnophilin underlines the utility of tetrahydropentalene derivatives in constructing complex natural products [5], demonstrating the structural and synthetic versatility of this class of compounds.

An in-depth review of the methodologies used for synthesizing tetrahydropentalene derivatives reveals a dynamic landscape marked by both tradition and novelty [1]. Each strategy offers unique advantages, such as enhanced yields and selectivity, yet often contends with the need for specialized reagents and stringent conditions, reflecting the evolving sophistication in the tetrahydropentalene synthesis.

However, it is safe to consider readily available tetrahydropentalene-2,5-dione (2,5-THP-dione) as the foundational material for the synthesis of tetrahydropentalene-based building blocks. Besides the fact that 2,5-THP-dione is a commercial bulk chemical, in our previous studies on the synthesis of bis-nor adamantane (stellane) and nor-adamantane derivatives [6, 7] we managed to adjust the literature protocol [8] for 2,5-THP-dione multikilo preparation. By leveraging the readily available and chemically versatile nature of 2,5-THP-dione, we devised a scalable synthesis approach facilitating the production of these compounds at multigram scales with yields ranging from good to excellent. Our study not only confirms the integral role of 2,5-THP-dione in fostering the synthesis of complex molecular structures, but also enhances the arsenal available for drug discovery and synthetic chemistry. Our efforts delineate a pathway for exploiting a broader spectrum of therapeutic and synthetic opportunities presented by tetrahydropentalene derivatives in the realm of medicinal chemistry and drug development.

Results and discussion

Armed with an ample supply of 2,5-THP-dione, we embarked on synthesizing derivatives poised for a significant impact in future derivatization, carefully considering aspects, such as heavy atom

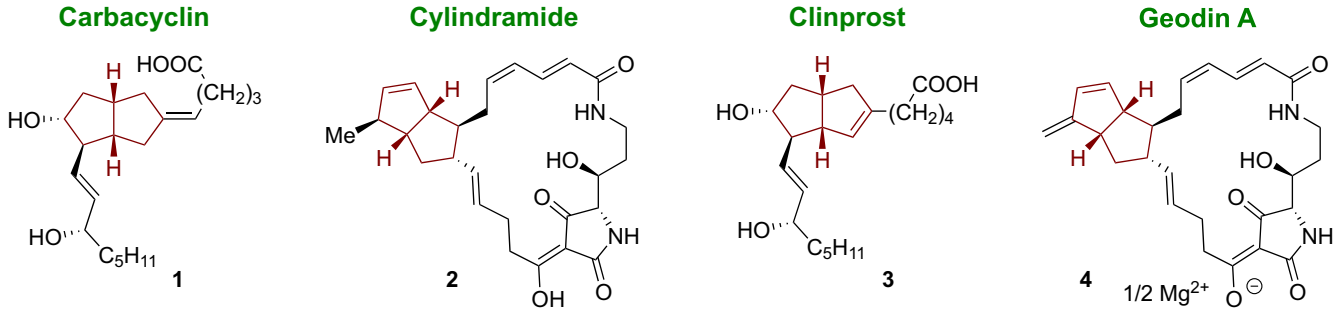


Figure. Some notable natural compounds with tetrahydropentalene core: carbocyclin (**1**), cyinderamide (**2**), clinprost (**3**), geodin A (**4**)

ISSN 2308-8303 (Print) / 2518-1548 (Online)

count and functional group distribution. Our selection included both di- and mono-functionalized derivatives, each chosen for its potential to enrich the chemical space of medicinal chemistry-relevant molecules. This choice was driven by the aim to harness the most valuable derivatives for the subsequent transformation, with an eye towards efficiency and scalability – the tactics that notably diverges from the more traditional approaches documented in the literature.

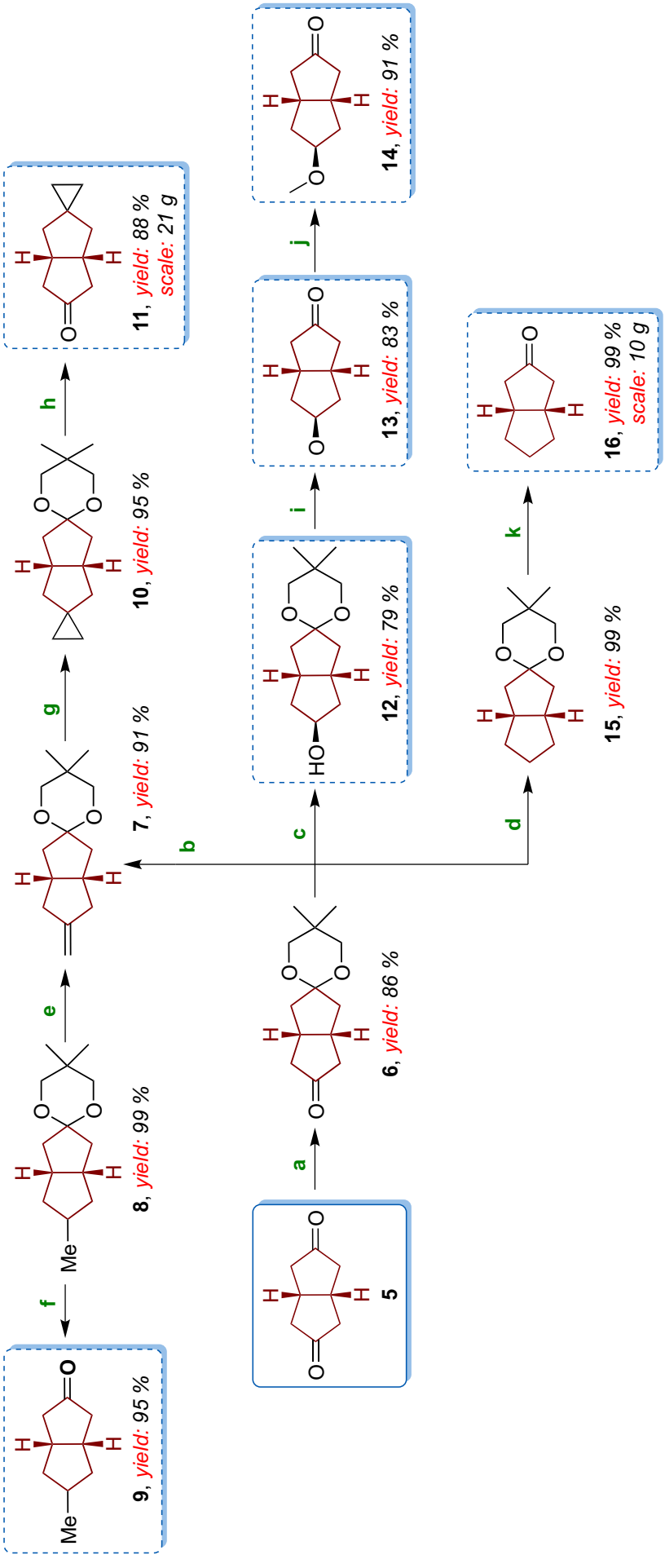
The synthetic studies began with compound **5**, going through its simple reaction with 2,2-dimethylpropanediol-1,3 under the PTSA catalysis in toluene to give compound **6** with a yield of 96% on the scale of over 40 grams per operation, thus simplifying the protocol and sidestepping the cumbersome separations that typically encumbered this transformation [9] (**Scheme**). The transition to compound **7** *via* reacting **6** with methylenetriphenylphosphorane "set the stage" for the synthesis of a suite of alkylated THP-ketones, including the previously unavailable methyl ketone **9** and tetrahydro-1'*H*-spiro[cyclopropane-1,2'-pentalen]-5'(3'*H*)-one **11** [10]. Methyl ketone **9** was synthesized through the series of transformations included a catalytic hydrogenation step utilizing palladium on activated charcoal under ambient conditions and the subsequent acid-mediated deprotection with a combined near-quantitative yield. Taking advantage of our previously developed in-flow diazomethane generation method [11], the transformation of compound **7** was carried out with diazomethane in the presence of palladium(II) acetate to obtain spiro-cyclopropane derivative **10** with a yield of 95%. It highlights this modern approach to the cyclopropanation of olefines and exhibits simplified and safer method for the synthesis of **10** compared to the previously described one [12]. The following acidic hydrolysis of compound **10** led to compound **11** with a yield of 88% on 22 g scale, further demonstrating the scalability and preparative potential of our synthesis strategies.

The next step was shifted to the transformation of compound **6**, which underscore the selective mono-reduction of the ketone group either to respective alcohol or hydrocarbon, opening opportunities for a broader THP core derivatization. The reduction of ketone **6** with the sodium borohydride powder in aprotic conditions (THF media) gave 12 grams of intermediate alcohol **12** with a yield of 79% (**Scheme**). The deprotection of compound **12** resulted in ketoalcohol **13** as a single diastereomer (*see* SI file for spectral details). The use of a standard methylation protocol made it possible to obtain methoxyketone **14** with a yield of 91%. Products **13** and **14** are valuable THP-derived building blocks as both are individual diastereomers with two separately derivatizable functional groups. The reduction of keto-group in **6** to -CH_2- was achieved using the standard Wolff–Kishner reduction [13] protocol with hydrazine hydrate in ethylene glycol, followed by the addition of potassium hydroxide, resulting in ketal **15**, which then was subjected to the acidic hydrolysis and yielded desired ketone **16** quantitatively across two steps (**Scheme**). In the case of product **16** we were able to achieve significantly elevated yields on a larger scale compared to the literature [14].

Our research successfully demonstrates the synthesis of a series of target tetrahydropentalene derivatives, including alkylated monofunctional compounds **9** and **11**, bifunctional derivatives **13** and **14**, and the monofunctional ketone **16**. Each compound, valuable in its unique way, represents a significant contribution to the field of medicinal chemistry and synthetic organic chemistry. By carefully modifying existing protocols, we have not only increased the efficiency of these syntheses, but also achieved them on a multigram scale, thereby demonstrating the scalability of our approaches. The high yields obtained for these compounds once again underline the effectiveness of our improved methodologies. It is also noteworthy that all the compounds synthesized resemble *cis*-configuration, which is typical for THP-derivatives, and additionally verified by 2D-NMR studies (*see SI file for details*) Taken together, these results highlight the potential of our building blocks synthesized for further derivatization and research within the THP-derived chemical space.

■ Conclusions

In our study of tetrahydropentalene-2,5-dione derivatives, inspired by the rich biological activity of natural bicyclo[3.3.0]octane compounds, we successfully synthesized a suite of alkylated and functional derivatives. Using commercially available 2,5-THP-dione as a key starting material, we streamlined the synthetic protocols, achieving scalable production with significant yields. This approach not only facilitated the preparation of the compounds important for medicinal chemistry, but also demonstrated the versatility of 2,5-THP-dione as a precursor. Our findings,



Scheme. The derivatization of tetrahydropentalene-2,5(1*H*,3*H*)-dione (**5**). *Experimental conditions:* (**a**) toluene/2,2-dimethyl-1,3-propanediol/*p*-TSA, 130 °C, 3 h; (**b**) THF/$Ph_3P{=}CH_2$, 0 °C to r.t., overnight; (**c**) THF/$NaBH_4$ (powder), -30 °C to r.t., overnight; (**d**) MEG/$NH_2NH_2{*}H_2O$, 90 °C, 2 h; KOH, 150 °C, 1 h; (**e**) MeOH, Pd/C (10 %), H_2, r.t., overnight; (**f**) THF/H_2O, HCl (conc.), LiCl, r.t., overnight; (**g**) DCM, $Pd(OAc)_2$, CH_2N_2, r.t., 1 h; (**h**) THF/H_2O, HCl (conc.), LiCl, r.t., overnight; (**i**) THF/H_2O, HCl (conc.), r.t., overnight; (**j**) MeCN, K_2CO_3, MeI, 40 °C, 18 h; (**k**) THF/H_2O, HCl (conc.), LiCl, r.t., overnight

ISSN 2308-8303 (Print) / 2518-1548 (Online)

marked by methodological innovation, efficiency and scalability, contribute significantly to the THP-derivatives pool available for wide derivatization and use in drug discovery projects, as well as demonstrate the essential role of tetrahydropentalenes in advancing synthetic chemistry and highlight their potential as valuable building blocks in the search for new therapeutic agents.

■ Experimental part

General information and materials

The solvents were purified according to the standard procedures. All starting materials were obtained from Enamine Ltd. Melting points were measured on the automated melting point system. NMR spectra were recorded on a Bruker Avance 500 spectrometer (at 500 MHz for ^{1}H and 126 MHz for ^{13}C nucleus) and Varian Unity Plus 400 spectrometers (at 400 MHz for ^{1}H, 101 MHz for ^{13}C nucleus). Tetramethylsilane (^{1}H, ^{13}C) was used as an internal standard. GCMS analyses were performed using an Agilent 5890 Series II 5972 GCMS instrument (electron impact (EI) ionization (70 eV)). Column chromatography was performed with silica gel (200-300 mesh). The elemental analysis was carried out in the Analytical Laboratory of the Institute of Organic Chemistry, NAS of Ukraine.

Experimental protocols

(3a*R*,6a*S*)-5',5'-Dimethyltetrahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxan]-5(3*H*)-one (6)

Compound **5** (30 g, 217.12 mmol), 2,2-dimethylpropanediol-1,3 (22.6 g, 217.12 mmol) and *p*-TSA (1.87 g, 0.05 equiv) were dissolved in toluene (500 mL). The mixture obtained was heated to 130 °C and stirred at this temperature with a Dean-Stark water trap. In 3 h it was cooled to room temperature and washed with Na_2CO_3 (200 mL, 10% aq. solution). The toluene layer was separated, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was purified by flash column chromatography (FCC) using EtOAc in hexane (0–50% gradient) as an eluent to give compound **6** as a colorless solid.

Yield – 41.7 g (86%). M. p. 42–46 °C. Anal. Calcd for $C_{13}H_{20}O_3$, %: C 69.61, H 8.99. Found, %: C 69.81, H 8.87. ^{1}H NMR (500 MHz, DMSO-d_6): δ, ppm: 0.86 (6H, s), 1.68–1.76 (2H, m), 1.95–2.03 (2H, m), 2.10–2.18 (2H, m), 2.33–2.44 (2H, m), 2.70 (2H, td, J = 8.7, 4.7 Hz), 3.36 (2H, s), 3.39 (2H, s). ^{13}C NMR (126 MHz, DMSO-d_6), δ, ppm: 22.1, 29.6, 36.0, 40.7, 44.0, 71.0, 71.1, 109.1, 219.0. GC-MS (EI), m/z: 224.2 $[M]^{+\cdot}$.

(3a*R*,6a*S*)-5',5'-dimethyl-5-methylenehexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxane] (7)

Compound **6** (41.7 g, 185.91 mmol) was dissolved in THF (300 mL) and added in a dropwise manner to a suspension of freshly prepared methylenetriphenylphosphorane (made from 122.7 g of methyltriphenylphosphonium iodide (1.5 equiv) cooled to 0 °C and 31.3 g of potassium *tert*-butoxide (1.5 equiv)). After the addition was complete, the resulting mixture was allowed to reach room temperature and left to stir at the given conditions overnight. Volatiles were removed *in vacuo*, the residue was treated with water (300 mL) and extracted with MTBE (3×250 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was passed through SiO_2 pad using MTBE as an eluent to give a crude compound **7** as a yellow oil.

Yield – 37.4 g (91%). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.93 (8H, d, J = 7 Hz), 1.41–1.53 (3H, m), 1.96–2.04 (3H, m), 2.04–2.10 (1H, m), 2.22–2.34 (2H, m), 2.41 (2H, s), 2.47–2.57 (2H, m), 3.44 (dd, J = 16.9, 6.7 Hz, 5H), 4.79 (2H, s).

(3a*R*,6a*S*)-5,5',5'-trimethylhexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxane] (8)

Compound **7** (5 g, 22.3 mmol) was dissolved in MeOH. Pd on activated charcoal (0.5 g, 10%) was added and the mixture obtained was evacuated and backfilled with hydrogen. The resulting suspension was stirred overnight at ambient temperature under H_2 atmosphere (balloon pressure). After that, the catalyst was filtered off, the filtrate was concentrated *in vacuo*, and the residue was used in further transformations with no additional treatment. ^{1}H NMR of the material obtained was not characteristic due to the partial deprotection of ketone and complexity of the spectrum. The yield was calculated as quantitive, ~5.1 g.

(3a*R*,6a*S*)-5-methylhexahydropentalen-2(1*H*)-one (9)

Compound **8** (5.1 g, the material from the previous step) was dissolved in a 1:1 mixture of THF (100 mL) and water (100 mL). Concentrated HCl (10 mL, 37% aq.), followed by 0.91 g of LiCl (22 mmol) was added. After the addition, the solution was allowed to stir at ambient temperature overnight. Volatiles were removed *in vacuo*. The residue was washed with MTBE (3×150 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was purified *via* FCC using MTBE in hexane (0–100% gradient) as an eluent to give compound **9** as a yellow oil.

Yield – 3.1 g (95%). Anal. Calcd for $C_9H_{14}O$, %: C 78.21, H 10.21. Found, %: C 78.32, H 10.27. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.79–1.56 (6H, m), 1.56–2.22 (6H, m), 2.40–2.56 (2H, m), 2.61–2.87 (2H, m). ^{13}C NMR (101 MHz, $CDCl_3$) δ, ppm: 19.9, 20.0, 36.3, 38.9, 39.7, 42.4, 43.0, 44.9, 45.3, 221.2. GC-MS (EI), *m/z*: 138.1 $[M]^{+\cdot}$.

(3a'*R*,6a'*S*)-5'',5''-Dimethyltetrahydro-1'*H*,3'*H*-dispiro[cyclopropane-1,2'-pentalene-5',2''-[1,3]dioxane] (10)

Compound **7** (37.4 g, 168.2 mmol) was dissolved in a solution of diazomethane (*ca.* 1 M) in DCM (250 mL). The mixture obtained was stirred with $Pd(OAc)_2$ (0.1 equiv) overnight. After a careful decomposition of the excessive diazomethane, it was concentrated *in vacuo* and passed through SiO_2 pad using MTBE as an eluent to give a crude compound **10** as a colorless oil used in the further step with no additional purification.

Yield – 35.7 g (*ca.* 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 0.29–0.52 (m, 8H), 0.94 (s, 6H), 1.27–1.30 (3H, m), 1.42–1.46 (3H, m), 1.62–1.82 (3H, m), 1.91–1.96 (3H, m), 2.17–2.22 (3H, m), 2.35 (1H, s), 2.47–2.63 (5H, m), 2.87 (3H, br. s), 3.49–3.51 (4H, m).

(3a'*R*,6a'*S*)-tetrahydro-1'*H*-spiro[cyclopropane-1,2'-pentalen]-5'(3'*H*)-one (11)

Compound **10** (35.7 g, 160 mmol) was dissolved in a 1:1 mixture of THF (300 mL) and water (300 mL). Concentrated HCl (30 mL, 37% aq.), followed by 6.7 g of LiCl (160 mmol) was added. After the addition, the mixture was allowed to stir at ambient temperature overnight. Volatiles were removed *in vacuo*; the residue was washed with MTBE (3×250 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was purified *via* FCC using MTBE in hexane (0-100% gradient) as an eluent to give compound **11** as a yellow oil.

Yield – 21.1 g (88%). Anal. Calcd for $C_{10}H_{14}O$, %: C 79.96, H 9.39. Found, %: C 80.11, H 9.30. ^{1}H HNMR (500 MHz, $CDCl_3$), δ, ppm: 0.36–0.44 (2H, m), 0.45–0.53 (2H, m), 1.41 (2H, dd, J = 13.1, 4.4 Hz), 1.91 (2H, dd, J = 13.1, 7.8 Hz), 2.10–2.21 (2H, m), 2.48 (2H, ddd, J = 19.2, 7.3, 2.2 Hz), 2.86 (2H, tt, J = 8.7, 4.7 Hz). ^{13}C NMR (151 MHz, $CDCl_3$), δ, ppm: 11.2, 12.9, 22.1, 40.3, 42.6, 44.8, 220.9. GC-MS (EI), *m/z*: 150.1 $[M]^{+\cdot}$.

(3a*R*,5r,6a*S*)-5',5'-dimethylhexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxan]-5-ol (12)

Compound **6** (15 g, 66.87 mmol) was dissolved in THF (300 mL), and this solution was cooled to -20–-30 °C. Then, dry $NaBH_4$ (2.54 g, 66.87 mmol) was added in portions, maintaining the internal temperature below -20 °C. After that, the mixture was allowed to slowly warm to room temperature and left to stir at the given conditions overnight. Then volatiles were removed *in vacuo*, the residue was treated with NH_4Cl (200 mL, 15% aq. solution) and extracted with MTBE (3×250 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Compound **12** was obtained as a white solid.

Yield – 11.86 g (79%). M. p. 61–62 °C. Anal. Calcd for $C_{13}H_{22}O_3$, %: C 68.99, H 9.80. Found, %: C 69.08, H 9.85. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.82–1.11 (6H, m), 1.44–1.62 (2H, m), 1.86–1.97 (3H, m), 2.03–2.31 (4H, m), 2.44–2.84 (2H, m), 3.40–3.58 (4H, m), 4.14–4.46 (1H, m). ^{13}C NMR (101 MHz, $CDCl_3$), δ, ppm: 21.32, 22.51, 30.07, 36.44, 37.76, 38.56, 40.80, 42.45, 42.93, 45.56, 71.59, 71.91, 72.18, 74.72, 75.58, 110.48. GC-MS (EI), *m/z*: 226 $[M]^{+\cdot}$.

(3a*R*,5r,6a*S*)-5-hydroxyhexahydropentalen-2(1*H*)-one (13)

Compound **12** (11.86 g, 52.47 mmol) was dissolved in a 1:1 mixture of THF (100 mL) and water (100 mL) and concentrated HCl (15 mL, 37%, aq.). After the complete dissolution, the mixture was allowed to stir at ambient temperature overnight. Volatiles were removed *in vacuo*; the residue was washed with EtOAc (5×75 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was purified *via* FCC using MTBE in hexane (0–100% gradient) as an eluent to give compound **13** as a white solid.

Yield – 6.12 g (83%). M. p. 50–52 °C. Anal. Calcd for $C_8H_{12}O_2$, %: C 68.55, H 8.63. Found, %: C 68.43, H 8.72. ^{1}H NMR (400 MHz, DMSO-d_6), δ, ppm: 1.38 (2H, dt, J = 13.2, 4.4 Hz), 1.96 (2H, ddd, J = 13.4, 8.2, 5.6 Hz), 2.07 (2H, dd, J = 18.9, 3.4 Hz), 2.38–2.42 (1H, m), 2.42–2.46 (1H, m), 2.59–2.73 (2H, m), 4.07–4.17 (1H, m), 4.51 (1H, d, J = 3.4 Hz). ^{13}C NMR (101 MHz, DMSO-d_6), δ, ppm: 37.6, 43.0, 45.6, 73.4, 220.2. GC-MS (EI), *m/z*: 140.1 $[M]^{+\cdot}$.

(3a*R*,5r,6a*S*)-5-methoxyhexahydropentalen-2(1*H*)-one (14)

Compound **13** (2 g, 14.3 mmol) was dissolved in MeCN (40 mL). Potassium carbonate (2.92 g, 21.45 mmol) and methyl iodide (3.05 g, 21.45 mmol) were added sequentially. The mixture obtained was stirred for 18 h at 40 °C. Then, it was cooled to room temperature, the insoluble substances were filtered off, and the filter cake was washed with an additional MeCN (10 mL) portion. The filtrate

ISSN 2308-8303 (Print) / 2518-1548 (Online)

was concentrated *in vacuo*, and the residue was passed through SiO_2 pad using MTBE as an eluent. The filtrate was concentrated *in vacuo* to give compound **14** as a colorless oil.

Yield – 2.01 g (91%). Anal. Calcd for $C_9H_{14}O_2$, %: C 70.10, H 9.15. Found, %: C 70.17, H 9.22. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.65 (2H, dt, J = 13.8, 4.0 Hz), 2.02–2.15 (2H, m), 2.21 (2H, dd, J = 19.3, 3.7 Hz), 2.43–2.55 (2H, m), 2.71–2.81 (2H, m), 3.24 (3H, s), 3.83–3.93 (1H, m). ^{13}C NMR (101 MHz, $CDCl_3$), δ, ppm: 37.74, 39.19, 45.25, 56.39, 83.61, 220.46. GC-MS (EI), m/z: 154.1 $[M]^{+\cdot}$.

(3a*R*,6a*S*)-5',5'-dimethylhexahydro-1*H*-spiro[pentalene-2,2'-[1,3]dioxane] (15)

Compound **6** (10 g, 44.54 mmol) was dissolved in ethylene glycol (40 mL) and treated with 4 equiv of hydrazine hydrate (8.9 g). This mixture was stirred for 2 h at 90 °C and then treated with KOH (9.98 g, 178.16 mmol, 4.0 equiv). The mixture obtained was heated to 150 °C and stirred at the given conditions for 1 h (strong N_2 evolution was observed). Then, it was cooled to room temperature and diluted with water (100 mL). The aqueous mixture was extracted with MTBE (3×100 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a crude product (the mixture contained the desired product, ethylene glycol and trace of the deprotected product). This mixture was then used with no additional treatment directly at the next step, the yield was calculated as close to quantitative.

(3aR,6aS)-hexahydropentalen-2(1*H*)-one (16)

A mixture containing compound **15** from the previous step was dissolved in a 1:1 solution of THF (100 mL) and water (100 mL) and concentrated HCl (15 mL, 37% aq.). After the complete dissolution of **15**, the mixture obtained was allowed to stir at ambient temperature overnight. Volatiles were removed *in vacuo*, and the residue was washed with Et_2O (3×100 mL). Combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A crude material was purified *via* FCC using MTBE in hexane (0–100% gradient) as an eluent to give compound **16** quantitatively as a colorless oil.

Anal. Calcd for $C_8H_{12}O$, %: C 77.38, H 9.74. Found, %: C 77.27, H 9.70. ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 1.33–1.46 (2H, m), 1.55–1.82 (2H, m), 1.85–2.06 (4H, m), 2.41–2.54 (2H, m), 2.58–2.76 (2H, m). ^{13}C NMR (101 MHz, $CDCl_3$), δ, ppm: 25.5, 33.4, 39.6, 44.7, 221.2. GC-MS (EI), m/z: 124.1 $[M]^{+\cdot}$.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)

Journal of Organic and Pharmaceutical Chemistry

Original Research

OPEN ACCESS
Freely available online on
http://ophcj.nuph.edu.ua

UDC 615.322:582.29

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The Study of Compounds Distilled with Water Vapor in *Cetraria islandica* (L.) Ach. Thalli Harvested in Ukraine

Abstract

The component composition and the quantitative content of compounds distilled with water vapor in the thalli of *Cetraria islandica* (L.) Ach. harvested in Ukraine were determined using the method of gas chromatography with mass spectrometric detection (GC/MS). 24 compounds distilled with water vapor were identified with the prevalence of fatty acids and their derivatives (57.86 ± 2.00 % of the total compounds), terpenoids and their derivatives (23.57 ± 0.97 % of the total compounds) and acyclic saturated hydrocarbons (10.99 ± 0.45 % of the total compounds). The highest percentage was observed for octadecadienoic (linoleic) acid (20.08 ± 0.67 % of the total compounds), hexadecanoic (palmitic) acid (19.21 ± 0.77 % of the total compounds) and 9,17-octadecadienal (18.57 ± 0.56 % of the total compounds). The presence of 4 monoterpenoids and 6 sesquiterpenoids in the raw material studied was determined for the first time.

Keywords: *Cetraria islandica*; thalli; compounds distilled with water vapor; GC/MS

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Дослідження сполук, дистильованих з водяною парою, що наявні у *Cetraria islandica* (L.) Ach. слані, заготовленої в Україні

Анотація

За допомогою методу газової хроматографії з мас-спектрометричним детектуванням (ГХ/МС) визначено компонентний склад та кількісний вміст дистильованих з водяною парою сполук, що наявні у слані *Cetraria islandica* (L.) Ach., заготовленої в Україні. Ідентифіковано 24 сполуки, дистильовані з водяною парою, серед яких переважали жирні кислоти та їхні похідні (57,86 ± 2,00 % від суми сполук), терпеноїди та їхні похідні (23,57 ± 0,97 % від суми сполук), ациклічні насичені вуглеводні (10,99 ± 0,45 % від суми сполук). Найвищий відсотковий вміст виявлено для октадекадієнової (лінолевої) кислоти (20,08 ± 0,67 % від суми сполук), гексадеканової (пальмітинової) кислоти (19,21 ± 0,77 % від суми сполук) та 9,17-октадекадієнового альдегіду (18,57 ± 0,56 % від суми сполук). Уперше для досліджуваної сировини виявлено наявність 4 монотерпеноїдів та 6 сесквітерпеноїдів.

Ключові слова: *Cetraria islandica*; слань; сполуки, дистильовані з водяною парою; ГХ/МС

Citation: Shpychak, A. O.; Khvorost, O. P. The Study of Compounds Distilled with Water Vapor in *Cetraria islandica* (L.) Ach. Thalli Harvested in Ukraine. *Journal of Organic and Pharmaceutical Chemistry* **2023**, *21* (4), 51–57.
https://doi.org/10.24959/ophcj.23.299297
Received: *25 October 2023*; **Revised:** *11 November 2023*; **Accepted:** *15 November 2023*

Funding: The authors received no specific funding for this work.
Conflict of interests: The authors have no conflict of interests to declare.

■ Introduction

It is known from the literature that lichen raw material has been an important component of traditional medicine, has long been used as a food product for humans and livestock, a source of spices, dyes and fragrances in the cosmetic and perfume industry in many cultures, mainly in Europe, South Asia and North America [1–3].

The widespread use of lichens for medical purposes and in different industries is due to the presence of various groups of biologically active substances (BAS) in their composition, such as polysaccharides, lichen acids, amino acids, proteins,

fatty acids, vitamins, which have been the study subject of many scientific works [1, 4–6].

The group of compounds distilled with water vapor is not the main group of compounds that cause the pharmacological effect of the lichen raw material, but it is worth paying attention to it for deepening the knowledge about the qualitative composition and the quantitative content of the BAS groups of the medicinal raw material. Some representatives of this group of compounds, such as aliphatic acids and aromatic compounds, are synthesized in lichens in the same way as lichen substances – specific secondary metabolites, some of them are unique to lichens and rarely occur in higher plants or fungi [1]. According to some data, the number of secondary metabolites found in lichens exceeds 1000 and includes aliphatic and aromatic acids, depsides, depsidones, dibenzofurans [3, 7]. Their presence causes antibacterial, antifungal, antiviral, antiproliferative, antioxidant, and other types of pharmacological activity [2, 3, 7, 8].

It has been demonstrated that essential oils obtained from lichens of the genus *Evernia* (*Parmeliaceae*) and *Ramalina* (*Ramalinaceae*), which include, in particular, such substances as *β*-pinene, *α*-pinene, camphene, limonene, myrcene, *α*-phellandrene, *p*-cymene, have antimicrobial and antifungal effects [7, 9].

One of the most known representatives of the *Parmeliaceae* family, *Cetraria islandica* (L.) Ach. has been used in folk medicine in Central and Northern Europe for centuries [10]. In pharmacognostic texts and handbooks of folk medicine, there are examples of the use of this lichen as an ingredient for the dishes of traditional cuisine and food supplements in case of the gastrointestinal tract disorders and for the treatment of respiratory diseases [2, 11].

Nowadays thalli of *C. islandica* are the pharmacopoeial medicinal raw material in many countries; they are applied to manufacture drugs with the proven efficiency used in the treatment of the respiratory system inflammatory processes, contribute to the regulation of respiratory organs, have antibacterial, anti-inflammatory, antitussive, softening and antioxidant effects [8, 12, 13].

Using gas chromatography with mass spectrometric detection it was possible to detect 23 compounds in the raw material of *C. islandica*, 16 of them were identified. Their composition included fatty acids, aldehydes, phenols, and their ethers, olefins, and lichen acids [14].

Since we have found only fragmentary data on the component composition of compounds distilled with water vapor in the thalli of *C. islandica* in the literature available to us, the study of this group of BAS as a part of a comprehensive phytochemical study of the raw material of *C. islandica* harvested in Ukraine is expedient, relevant and has practical importance for the development of modern effective medicinal products.

The aim of this work was to study the component composition and determine the quantitative content of compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine to deepen knowledge about the qualitative composition and quantitative content of BAS in the domestic raw material and for further obtaining new substances and medicinal products based on it.

■ Materials and methods

Plant raw material

For the study, thalli of *C. islandica* harvested in late summer/early autumn 2019 in the territory of the Rakhiv district of the Zakarpattia region were used. The raw material was dried in the open air under a cover to an air-dry condition and stored in paper bags in a dry place protected from direct sunlight.

Equipment and conditions of chromatographic separation

The determination of the component composition of compounds distilled with water vapor in the raw material was carried out by the method of gas chromatography with mass spectrometric detection (GC/MS) [15, 16].

The chromatographic separation was performed on an Agilent 6890N gas chromatograph with a 5973 inert mass detector (Agilent Technologies, USA). A HP-5ms capillary column (length – 30 m, inner diameter – 0.25 mm, diameter of the sorbent grain – 0.25 μm) was used. The separation was carried out in the gradient mode. The initial temperature of 50 °C was maintained for 5 min, then increased to 220 °C with the rate of 4 °C per minute, then with the rate of 10 °C to 300 °C was maintained for 10 min. The flow rate of the carrier gas (helium) through the column was 1.0 mL min^{-1}. The temperature of the evaporator was 300 °C, the sample was injected in a split mode with the rate of 1:50, and the injection volume was 2 μL. The detection was carried out in the SCAN mode in the range of 38–400 m/z [17, 18].

Preparation of the raw material sample

The raw material previously crushed with a laboratory mill LGM-1 (Olis, Ukraine) was ground

ISSN 2308-8303 (Print) / 2518-1548 (Online)

to a powdery state in a glass mortar. After that, 300 mL of water was added to 5.00 g (accurate weight) of the raw material and refluxed at a temperature of 100 °C for 3 h. The distilled water was extracted with heptane. The extract was evaporated to 100–200 µL in a stream of nitrogen [19].

Identification and quantification

The identification of compounds distilled with water vapor in the samples studied was performed using the NIST 02 mass spectrum library. The match percentage of the compounds detected with the compounds from the NIST 02 mass spectrum library was 80–99% [20, 21].

The quantitative content (%) of the total compounds was calculated by comparing the peak area of the components with the sum of the areas of all peaks on the chromatogram [15, 17].

■ Results and discussion

The GC/MS chromatogram obtained for compounds distilled with water vapor is shown in **Figure**. The results of the determination of the component composition and the quantitative content of compounds distilled with water vapor in the raw material of *C. islandica*, as well as their chromatographic parameters, are given in **Table**.

As a result of the study, 24 compounds distilled with water vapor were identified in the thalli of *C. islandica* harvested in Ukraine, including terpenoids and their derivatives, acyclic saturated hydrocarbons, dienes, fatty acids, esters.

Some of the compounds identified (2,2,4,4,6,6-hexamethyl-1,3,5,2,4,6-trioxatrisilinane, diisobutyl phthalate, 5-methyl-2-phenylindolizine) probably could have entered the samples studied from the outside during harvesting or transportation.

A significant part of the substances identified were terpenoids represented by 10 compounds and made up 23.57 ± 0.97% of the total quantitative content of compounds distilled with water vapor. Among them, 3 monocyclic monoterpenoids (*cis*-menthone, *trans*-menthone, menthol), bicyclic monoterpenoid *trans*-carane and 6 sesquiterpenoids (caryophyllene, *β*-cubebene, *γ*-muurolene, δ-amorphene, sesquiterpene ketone mayurone, hexahydrofarnesyl acetone) were found.

Among the compounds detected, the highest quantitative content was observed for polyunsaturated ω-6 fatty octadecadienoic (linoleic) acid – 20.08 ± 0.67% of the total compounds, saturated *n*-hexadecanoic (palmitic) acid – 19.21 ± 0.77% of the total compounds and 9,17-octadecadienal – 18.57 ± 0.56% of the total compounds.

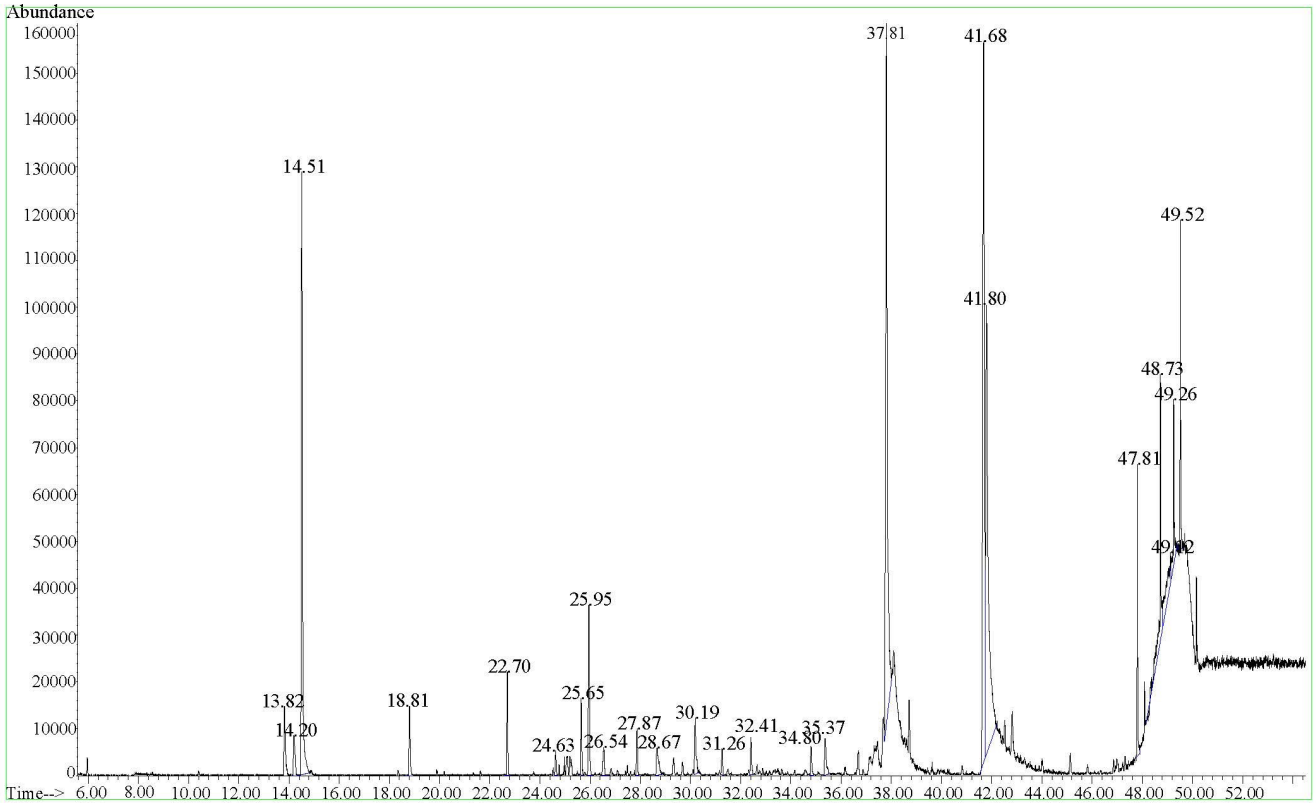


Figure. The GC/MS chromatogram of compounds distilled with water vapor in the thalli of *C. islandica* harvested in Ukraine

Table. The component composition and the quantitative content of compounds distilled with water vapor in the thalli of *C. islandica* (n = 5)

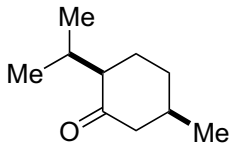
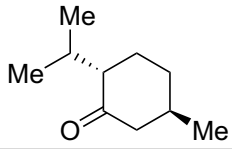
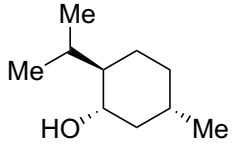
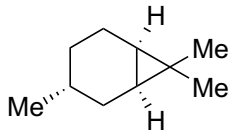
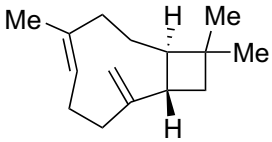
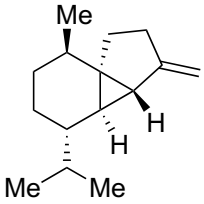
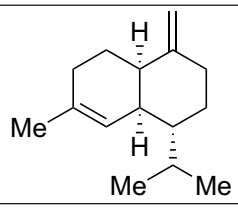
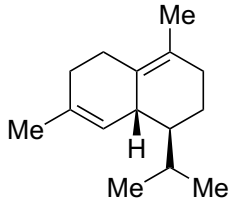
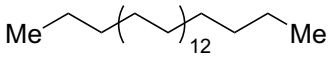
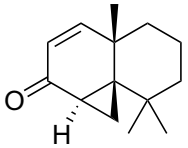
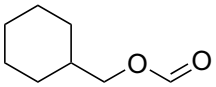
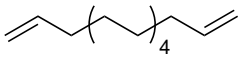
Retention time, min	The structure of the compound	The name of the compound	Content, %
1	2	3	4
13.83		*cis*-menthone	1.59 ± 0.04
14.20		*trans*-menthone	1.03 ± 0.05
14.51		menthol	11.95 ± 0.49
18.80		*trans*-carane	1.27 ± 0.06
22.70		caryophyllene	1.73 ± 0.08
24.63		*β*-cubebene	0.38 ± 0.02
25.65		*γ*-muurolene	1.27 ± 0.06
25.95		(+)-*δ*-amorphene	2.99 ± 0.12
26.55		hentriacontane	0.69 ± 0.03
27.87		mayurone	0.79 ± 0.02
28.67		cyclohexylmethyl formate	0.98 ± 0.04
30.19		1,13-tetradecadiene	1.24 ± 0.06

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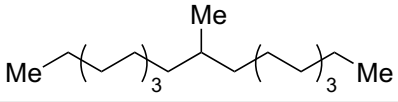
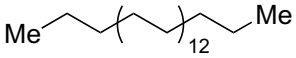
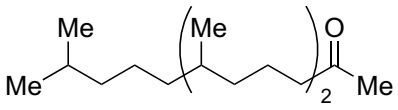
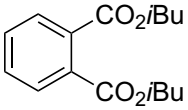
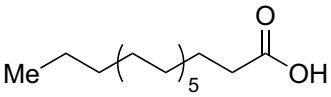
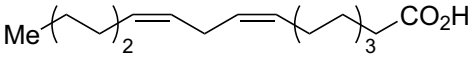
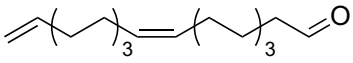
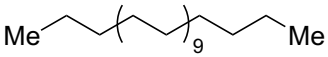
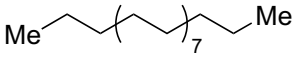
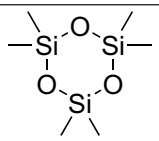
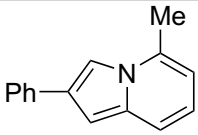
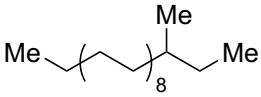
Table *(continued)*

1	2	3	4
31.26		10-methylnonadecane	0.47 ± 0.02
32.41		triacontane	0.68 ± 0.03
34.80		6,10,14-trimethylpentadecan-2-one	0.57 ± 0.03
35.37		diisobutyl phthalate	0.97 ± 0.04
37.81		palmitic acid	19.21 ± 0.77
41.68		linoleic acid	20.08 ± 0.67
41.80		9,17-octadecadienal	18.57 ± 0.56
47.81		pentacosane	2.38 ± 0.12
48.73		eicosane	3.99 ± 0.15
49.13		2,2,4,4,6,6-hexamethyl-1,3,5,2,4,6-trioxatrisilinane	2.28 ± 0.11
49.25		5-methyl-2-phenylindolizine	2.13 ± 0.8
49.52		3-methylheneicosane	2.78 ± 0.10

Six acyclic saturated hydrocarbons (10.99 ± 0.45% of the total compounds) were found in the raw material, namely hentriacontane, 10-methylnonadecane, triacontane, pentacosane, eicosane, 3-methyl-heneicosane, among them eicosane (3.99 ± 0.15% of the total compounds) dominated.

Such groups of BAS as fatty acid derivatives, aldehydes and dienes, which were detected as a result of our study, were also found in the results of the previous studies of the raw material of *C. islandica* conducted by the GC/MS method [14]. However, these researchers did not mention the presence of terpenoids and their derivatives in the raw material studied. Therefore, there is no data on the presence of *cis*-menthone, *trans*-menthone, menthol, *trans*-carane, caryophyllene, *β*-cubebene, *γ*-murolene, *δ*-amorphene, mayurone, hexahydrofarnesyl acetone; these data are provided for the first time.

According to literature data, phenols and their ethers, aldehydes, and lichen acids dominated among the compounds identified in the raw material, while according to the results of our study, fatty acids and their derivatives prevailed in percentage.

The presence of terpenoids and their derivatives, aldehydes in the raw material studied suggests that these compounds play a certain role in the anti-inflammatory, antiseptic, and expectorant pharmacological activity, which should be taken into account when developing new substances and medicinal products based on them.

■ Conclusions

The component composition of compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine has been studied by the GC/MS method. 24 compounds distilled with water vapor have been identified; among them fatty acids and their derivatives (57.86 ± 2.00% of the total compounds), terpenoids and their derivatives (23.57 ± 0.97% of the total compounds) and acyclic saturated hydrocarbons (10.99 ± 0.45% of the total compounds) predominate. For the first time, the presence of 4 monoterpenoids and 6 sesquiterpenoids has been determined in the raw material studied. The results obtained regarding the compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine indicate the need for further research, considering the batches of the raw material and places of harvesting, possibly in comparison with the raw material harvested outside the country.

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ISSN 2308-8303 (Print) / 2518-1548 (Online)