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SHORT COMMUNICATION

Synthesis of some spiro[chromane-2,3′-pyrrolidine]-4-one derivatives and investigation of their antifungal activity

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Abstract: Eleven spiro[chromane-2,3′-pyrrolidine]-4-one derivatives were synthesized, and their antifungal activity against *Candida albicans* and *Cryptococcus neoformans* was tested. Among synthesized derivatives, one antifungal-active substance was revealed, 6-fluorospiro[chromane-2,3′-pyrrolidine]-4-one hydrochloride inhibiting growth of *Cryptococcus neoformans* with the MIC value 16 μg/ml. This compound demonstrates no cytotoxicity or hemolytic activity against human cell line HEK-293 and red blood cells.

Keywords: spiro[chromane-2,3′-pyrrolidine]-4-ones; synthesis; antifungal activity; *Cryptococcus neoformans*.

Introduction

Antimicrobial resistance (AMR) has been recognized by the World Health Organization (WHO) as the one of the top global public health and development threats [1] that world encountered at the current stage of evolution of microorganisms being forced to survive under widespread overuse of antibiotics. AMR not just raises concern of spreading infectious diseases itself, but moreover, renders many of gains of modern medicine obsolete making many medical procedures and treatments such as surgery, caesarean sections and cancer chemotherapy much riskier.

One of the way of addressing AMR is through research and development of new antimicrobials. Aiming at focus these efforts on the crucial pathogens, WHO developed and published the “WHO fungal priority pathogen list” [2].

Cryptococcus neoformans and *Candida albicans* selected for our investigation are the pathogens from the most threatening Critical Priority Group of this list.

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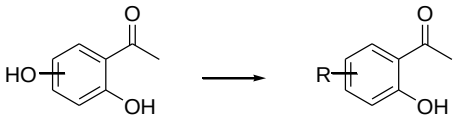
The spiro[chromane-2,3′-pyrrolidine]-4-one derivatives demonstrate various biological activity. They were developed as inhibitors of stearoyl-CoA-desaturase [3], acetyl-CoA-carboxylase (ACC) [4], histone deacetylase [5] and other targets, but never as antibiotics. At the same time their close relative homologs, spiro[chromane-2,4′-piperidine]-4-one derivatives recently were investigated as potential inhibitors of fatty acid synthesis in pathogenic microorganisms [6].

Here we are representing the synthesis of 11 novel spiro[chromane-2,3′-pyrrolidine]-4-one derivatives and evaluation of their antifungal activity.

Results and Discussion

All spiro[chromane-2,3′-pyrrolidine]-4-one derivatives were synthesized starting from the corresponding 2-hydroxyacetophenones. 2-Hydroxy-5-methoxyacetophenone (**1**), 5-benzyloxy-2-hydroxyacetophenone (**2**) and 5-buthoxy-2-hydroxyacetophenone (**3**) were synthesized by the alkylation of 2,5-dihydroxyacetophenone with correspondingly methyl iodide, benzyl chloride, and butyl iodide (Scheme 1) according to the approach [7]. 2-Benzyoloxy-6-hydroxyacetophenone (**4**) was obtained in the same way by reaction between 2,6-dihydroxyacetophenone and benzyl chloride [8].

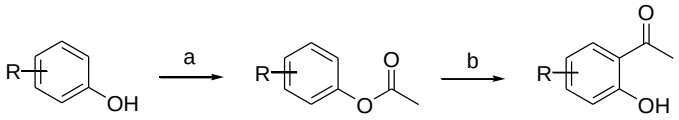
2-Hydroxy-5-fluoroacetophenone (**5**) was synthesized by acetylation 4-fluorophenol and following Fries rearrange-



1-4

R = 5-MeO (**1**); 5-BzlO (**2**); 5-BuO (**3**); 6-BzlO (**4**)

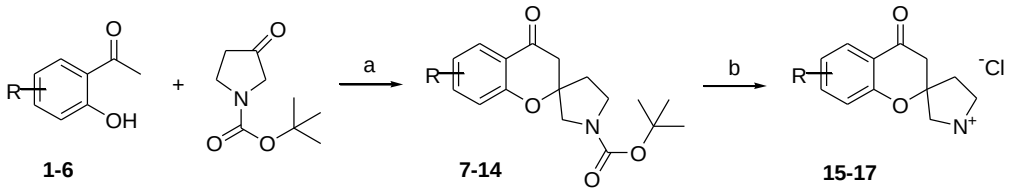
Scheme 1. Synthesis of 2-hydroxyacetophenones **1-4**. Conditions a) K_2CO_3, acetone, 6h, reflux.



5-6

R = 5-F (**5**); 3-Et, 5-Br (**6**)

Scheme 2. Synthesis of 2-hydroxyacetophenones **5-6**. Conditions a) CH_3COCl, 2h, r.t.; b) $AlCl_3$, 130 °C, 2h.



1-6 **7-14** **15-17**

R = 6-MeO (**7**); 6-Br, 8-Et (**8**); H, 6-BuO (**10**); 7-OH (**11**); 6-OH (**12**); 5-BzlO (**13**); 6-BzlO(**14**); 6-F (**15**); 6-MeO (**16**); H (**17**).

Scheme 3. Synthesis of spiro[chromane-2,3′-pyrrolidine]-4-one derivatives. Conditions a) MeOH, 200% mol. pyrrolidine, 4h, reflux; b) HCl/dioxane, 1h, r.t.

ment [9]. 2-Hydroxy-3-ethyl-5-bromoacetophenone (**6**) was obtained starting from 2-ethylphenol. The bromination process was carried out according to the method [10], which yielded 2-ethyl-5-bromophenol. Following acetylation, as described in [9], 2-ethyl-5-bromophenyl acetate was obtained. The Fries rearrangement in accordance with [9] leads finally to 2-hydroxy-3-ethyl-5-bromoacetophenone (**6**) (Scheme 2).

The corresponding Boc-protected spiro[chromane-2,3′-pyrrolidine]-4-one derivatives **7-14** were synthesized by the condensation Boc-pyrrolidine-3-one with 2-hydroxy-acetophenones in methanol solution catalyzed by pyrrolidine [5]. The Boc-protection removal was carried out using hydrogen chloride in dioxane giving amine hydrochlorides **15-17** according to the Scheme 3.

Antifungal activity of 11 novel spiro[chromane-2,3′-pyrrolidine]-4-one derivatives **7-17** was tested at the University of Queensland (Australia) in a framework of CO-ADD (The Community for Antimicrobial Drug Discovery), funded by the Wellcome Trust (UK) on pathogenic strains *Cryptococcus neoformans var. Grubii* ATCC 208821 and *Candida albicans* ATCC90028 [11]. Cytotoxicity and hemolysis assay of the synthesized compounds were determined using human embryonic kidney cells HEK-293 ATCC CRL-1573 and human red blood cells.

Ten of investigated compounds demonstrate no significant antifungal activity at the studied concentrations. At the same time 6-fluorospiro[chromane-2,3′-pyrrolidine]-4-one hydrochloride (**15**) showed promising antifungal activity against the *Cryptococcus neoformans* with MIC value reaching 16 μg/ml. This compound doesn't show toxicity against human cells used in testing.

Conclusions

Eleven novel spiro[chromane-2,3′-pyrrolidine]-4-one derivatives have been synthesized. One of them, namely 6-fluorospiro[chromene-2,3′-pyrrolidine]-4-one hydrochloride, exhibits antifungal activity against *Cryptococcus neoformans* with a minimum inhibitory concentration (MIC) value of 16 μg/ml and no cytotoxic or hemolytic activity against human cells, making it a promising compound for further studies in the field of structural optimization and identification of its molecular target in pathogenic fungi.

Experimental section

2-Hydroxyacetophenone, 2,4-dihydroxyacetophenone, 2,5-dihydroxyacetophenone, 2,6-dihydroxyacetophenone, 4-fluorophenol and 2-ethylphenol as well as Boc-pyrrolidine-3-one were purchased from Acros Organics BVBA. The structures of synthesized compounds have been confirmed by ^{1}H NMR spectra recorded on a Varian MercuryVRX-400 instrument with an operating frequency 400 MHz and an internal standard TMS (tetramethylsilane). High-performance liquid chromatography-mass spectrometric analysis (LC-MS) was performed on Agilent 1100LC/MSD SL instrument (Agilent Technologies, USA) equipped with Zorbax SB-C18 Rapid Resolution HT Cartridge (2.1×30 mm, 1.8 μm) using a 0-100% gradient (2 min) of CH_3CN in 0.1% formic acid.

Synthesis

1-(5-Butoxy-2-hydroxyphenyl)ethanone (***3***) as well as other 2-hydroxy-5- or 6-alkoxoacetophenones **1**-**4** were synthesized by the alkylation of dihydroxyacetophenones with corresponding alkyl halide following the procedure described if reference [7]. Yield: 62%. Light brown powder. M.p. 33-35 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.50 (s, 1H), 7.30 (d, J = 2.9Hz, 1H), 7.14 (dd, J = 8.8, 2.9 Hz, 1H), 6.88 (d, J = 8.8Hz, 1H), 3.93 (t, J = 6.6Hz, 2H), 2.63 (s, 3H), 1.67 (m, 2H), 1.43 (m, 2H), 0.93 (t, J = 7.3Hz, 3H). LC-MS m/z $(M+H)^+$ 209.

1-(5-Bromo-3-ethyl-2-hydroxyphenyl)ethanone (***6***) was synthesized as it's reported in [11]. Yield: 39%. Light yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.438 (s, 1H), 7.648 (s, 1H), 7.403 (s, 1H), 2.637 (m, 5H), 1.221 (t, J = 7.3Hz, 3H). LC-MS m/z $(M+H)^+$ 244.

General procedure for synthesis of spiro[chromane-2,3′-pyrrolidine]-4-one derivatives.

10 mmol of corresponding 2-hydroxyacetophenone, 1.85 g (10 mmol) of *N*-Boc pyrrolidine-3-one and 1.67 mL (20 mmol) pyrrolidine dissolved in 10 ml of MeOH were heated under reflux for 4 h. Then volatile material was evaporated off under vacuum and the crude products we purified by column chromatography (eluent: petroleum ether/EtOAc 95:5 to 7:3) to obtain 4-oxo-spiro(chromane-2,3′-pyrrolidine)-1′-carboxylic acid *tert*-butyl esters **7**-**14**.

*tert-Butyl 6-methoxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**7**).*

Yield: 57%. Brown powder. M.p. 115-117 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (d, J = 3.2Hz, 1H), 7.13 (dd, J = 8.8, 3.2 Hz, 1H), 6.92 (d, J = 3.2Hz, 1H), 3.77 (s, 3H), 3.60 (m, 1H), 3.494 (m, 1H), 3.40 (m, 1H), 2.96 (m, 2H), 2.20 (m, 1H), 1.964 (m, 1H), 1.41 (d, 9H). LC-MS m/z $(M+H)^+$ 334.

*tert-Butyl 6-bromo-8-ethyl-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**8**).*

Yield: 69%. White powder M.p. 93-95 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.43 (s, 1H), 3.83 (d, J = 11.7, 1H), 3.60 (m, 3H), 3.32 (m, 1H), 2.60 (m, 3H), 2.30 (m, 1H), 1.94 (m, 1H), 1.45 (s, 9H), 1.18 (t, J = 7.3 Hz, 3H). LC-MS m/z $(M-C_4H_9)^+$ 353.8.

*tert-Butyl 4-oxospiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**9**).*

Yield: 51%. Light brown powder. M.p. 106-108 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 7.3 Hz, 1H), 7.47 (m, 1H), 7.02 (m, 1H), 6.95 (d, J = 8.5 Hz, 1H), 3.75 (d, J = 12.9, 1H), 3.55 (m, 2H), 3.36 (t, J = 12.7 Hz, 1H), 2.92 (m, 1H), 2.78 (m, 1H), 2.30 (m, 1H), 1.90 (m, 1H), 1.46 (s, 9H). LC-MS m/z $(M-C_4H_8)^+$ 248.

*tert-Butyl 6-butoxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**10**).*

Yield: 54%. White powder M.p. 74-76 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H), 3.73 (dd, J = 12.7, 4.3 Hz, 1H), 3.53 (m, 2H), 3.43 (t, J = 12.9 Hz, 1H), 2.88 (dd, J = 17.1, 5.4 Hz, 1H), 2.75 (m, 1H), 2.29 (m, 1H), 1.88 (m, 1H), 1.75 (m, 2H), 1.47 (m, 11H), 0.99 (t, J = 7.3 Hz, 3H). LC-MS m/z $(M-C_4H_8)^+$ 320.

tert-Butyl 7-hydroxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (***11***).

Yield: 48%. White powder. M.p. 154-156 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.58 (d, J = 8.3 Hz, 1H), 6.44 (d, J = 8.3 Hz, 1H), 6.25 (s, 1H), 3.52 (m, 2H), 3.34 (m, 2H), 2.79 (m, 2H), 2.19 (m, 1H), 1.94 (m, 1H), 1.42 (s, 9H). LC-MS m/z $(M-C_4H_8)^+$ 264.

*tert-Butyl 6-hydroxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**12**).*

Yield: 50%. White powder M.p. 181-183 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H), 7.07 (s, 1H), 6.94 (m, 1H), 6.78 (m, 1H), 3.59 (m, 1H), 3.43 (m, 2H), 3.27 (m, 1H), 2.90 (m, 1H), 2.78 (m, 1H), 2.19 (m, 1H), 1.92 (m, 1H), 1.41 (s, 9H). LC-MS m/z $(M+H)^+$ 320.

*tert-Butyl 5-benzyloxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**13**).*

Yield: 54%. White powder M.p. 123-125 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (m, 2H), 7.32 (m, 4H), 6.54 (m, 2H), 5.17 (s 2H), 3.74 (m, 1H), 3.53 (m, 2H), 3.36 (t, J 12.5 Hz, 1H), 2.83 (m, 2H), 2.30 (m, 1H), 1.91 (m, 1H), 1.46 (s, 9H). LC-MS m/z $(M+H)^+$ 409.8.

*tert-Butyl 6-benzyloxy-4-oxo-spiro[chromane-2,3'-pyrrolidine]-1'-carboxylate (**14**).*

Yield: 56%. White powder M.p. 90-92 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 6H), 7.14 (m, 1H), 6.91 (d, J = 8.5 Hz, 1H), 5.03 (s, 2H), 3.75 (m, 1H), 3.54 (m, 2H), 3.34 (t, J = 11.5 Hz, 1H), 2.84 (m, 2H), 2.31 (m, 1H), 1.90 (m, 1H), 1.50 (s, 9H). LC-MS m/z $(M-C_4H_8)^+$ 353.8.

General procedure for Boc-deprotection.

3 ml of 4M dioxane solution of hydrogen chloride was added to solution of 3 mmol *Boc*-derivative in 9 ml of dry dioxane. The mixture was stirred at the room temperature until the starting material disappeared according to the TLC data. The solution was evaporated, triturated with MTBE, filtered off, washed with MTBE, and vacuum-dried. The yield of hydrochlorides was almost quantitative.

*6-Fluorospiro[chromane-2,3'-pyrrolidine]-4-one hydrochloride (**15**).*

Yield: 95%. Brown powder. M.p. 192-194 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 2H), 7.51 (m, 2H), 7.10 (dd, J = 8.7, 4.3 Hz, 1H), 3.52 (m, 1H), 3.27 (m, 1H), 3.18 (d, J = 17.1, 1H), 3.10 (d, J = 17.1, 1H), 2.30 (d, J = 17.1, 1H), 2.07 (m, 1H). LC-MS m/z $(M+H-HCl)^+$ 222.

*6-Methoxyspiro[chromane-2,3'-pyrrolidine]-4-one hydrochloride (**16**).*

Yield: 96%. Brown powder. M.p. 197-199 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 2H), 7.24 (dd, J = 8.8, 3.2 Hz, 1H), 7.20 (d, J = 3.2 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H),

3.76 (s, 3H), 3.50 (d, *J* = 12.7 Hz, 1H), 3.39 (m, 1H), 3.25 (d, *J* = 12.7 Hz, 1H), 3.13 (d, *J* = 17.1, 1H), 3.04 (d, *J* = 17.1, 1H), 2.29 (m, 1H), 2.04 (m, 1H). LC-MS m/z $(M+H-HCl)^+$ 234.

Spiro[chromane-2,3'-pyrrolidine]-4-one hydrochloride (***17***).

Yield: 92%. Light brown powder. M.p. 189-191 ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 2H), 7.77 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.62 (dt, *J* = *7*,3 1.4 Hz, 1H), 7.11 (t, *J* = 7.3 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 3.50 (d, *J* = 12.4 Hz, 1H), 3.34 (m, 3H), 3.17 (d, *J* = 17.1 Hz, 1H), 3.06 (d, *J* = 17.1 Hz, 1H), 2.28 (m, 1H), 2.04 (m, 1H). LC-MS m/z $(M+H-HCl)^+$ 204.

Activity studies

To determine the minimum antifungal inhibitory concentration (MIC), cytotoxicity (CC_{50}) on mammalian cells, and hemolytic activity (HC_{10}) on human erythrocytes, solutions of compounds were prepared by sequential two-fold dilutions (1:2) from 32 up to 0.25 μg/ml (or from 20 to 0.156 μM) of compounds tested.

Antimicrobial assays

Determination of antifungal activity of the newly synthesized compounds was carried out according to the standard protocols of the Community for Open Antimicrobial Drug Discovery laboratory at The University of Queensland (Australia). The minimal inhibitory concentrations (MIC) were found at which complete/partial inhibition of growth and reproduction of fungi was observed. Two reference test strains of yeast-like fungi (*Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 208821) were taken. The work was performed using 384-well non-binding polystyrene plates (Fisher Scientific). Such antimicrobial agents as amphotericin B and fluconazole served as references. *Cytotoxicity* and *Hemolysis assay* was carried out using HEK293 (human embryonic kidney cells, ATCC CRL-1573) for cytotoxicity and human blood erythrocytes (obtained from the Australian Red Cross Blood Service) for hemolysis assays according to known standard procedures.

Notes

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Синтез та дослідження протигрибкової активності низки похідних спіро[хроман-2,3′-піролідин]-4-ону

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Резюме: Синтезовано одинадцять похідних спіро[хроман-2,3′-піролідин]-4-ону та вивчено їх протигрибкову активність щодо *Candida albicans* та *Cryptococcus neoformans*. Знайдено, що сполука 6-флуороспіро[хромен-2,3′-піролідин]-4-он гідрохлорид активна проти *Cryptococcus neoformans* та характеризується MIC 16 мг/мл. Водночас, ця речовина не виявляє цитотоксичної чи гемолітичної активності стосовно клітинної лінії HEK-293 та еритроцитів людини.

Ключові слова: спіро[хроман-2,3′-піролідин]-4-они; синтез; протигрибкова активність; *Cryptococcus neoformans.*