СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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The synthesis and the antimicrobial activity of *N*^{1}-substituted 5-amino-4-arylsulfonyl-3-*N*-phenylaminopyrazoles

This article is continuation of the development of methods for the synthesis of small molecules based on the structure of 5-aminopyrazole. The synthesis and the antimicrobial activity for a series of new *N*^{1}-subsituted 5-amino-4-arylsulfonyl-3-*N*-phenylaminopyrazoles have been described.

Aim. To synthesize derivatives of 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles and study their antimicrobial and antifungal properties.

Materials and methods. The methods of organic synthesis, instrumental methods of organic compounds analysis and methods of microbiological screening were used.

Results and discussion. 5-Amino-4-arylsulfonyl-3-phenylaminopyrazoles were prepared by the reaction of arylsulfonylacetonitriles with isothiocyanates in the presence of NaOH and CH_3I with further cyclization with hydrazine hydrate. The reaction of this compounds with *N*-arylchloroacetamides finished a series of *N*^{1}-substituted 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles. The antibacterial and antifungal properties of the compounds synthesized were studied. Some of the compounds obtained appeared to be potent inhibitors for several pathogenic bacterial and fungal lines.

Conclusions. The synthetic scheme for obtaining of *N*^{1}-substituted 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles, which can be used for creation of a library of compounds for *in vitro* antimicrobial screening, has been proposed. Some of the compounds synthesized are of certain interest as potential pharmaceutical agents and can be used to develop new antifungal agents.

Key words**:** *5-aminopyrazole; synthesis; antimicrobial activity; antifungal activity*

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Синтез та антимікробна активність *N*^{1} заміщених 5-аміно-4- арилсульфоніл-3-*N*-феніламінопіразолів

Стаття є продовженням розробки методів синтезу малих молекул на основі базової структури 5-амінопіразолу. Представлено синтез та вивчення антимікробної активності ряду нових *N*^{1}-заміщених 5-аміно-4-арилсульфоніл-3-*N*-феніламінопіразолів.

Мета роботи. Здійснити синтез похідних 5-аміно-4-арилсульфоніл-3-феніламінопіразолів та вивчити їх антимікробні та протигрибкові властивості.

Матеріали та методи. Методи органічного синтезу, інструментальні методи аналізу органічних сполук, методи мікробіологічного скринінгу.

Результати та їх обговорення. При взаємодії арилсульфонілацетонітрилів з ізотіоціанатами в присутності NaOH і CH_3I з подальшою циклізацією під дією гідразин-гідрату одержано 5-аміно-4-арилсульфоніл-3-феніламінопіразоли. В ході реакції цих сполук з *N*-арилхлорацетамідами синтезовано ряд *N*^{1}-заміщених 5-аміно-4-арилсульфоніл-3-феніламінопіразолів. Вивчені антимікробні та протигрибкові властивості синтезованих сполук, деякі з них є потенційними інгібіторами патогенних бактерій і грибів.

Висновки. Запропоновано синтетичну схему одержання *N*^{1}-заміщених 5-аміно-4-арилсульфоніл-3-феніламінопіразолів, придатну для створення бібліотеки сполук для антимікробного скринінгу *in vitro*. Деякі синтезовані сполуки становлять певний інтерес як потенційні фармацевтичні агенти і можуть бути використані для розробки нових протигрибкових агентів.

Ключові слова**:** *5-амінопіразол; синтез; антимікробна активність; протигрибкова активність*

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Синтез и противомикробная активность N^1 замещенных 5-амино-4-арилсульфонил-3-*N*-фениламинопиразолов

Работа является продолжением разработки методов синтеза малых молекул с базовой структурой 5-аминопиразола. Описан синтез и изучение противомикробной активности ряда новых N^1-замещенных 5-амино-4-арилсульфонил-3-*N*-фениламинопиразолов.

Цель работы. Осуществить синтез производных 5-амино-4-арилсульфонил-3-фениламинопиразолов и изучить их противомикробные и противогрибковые свойства.

Материалы и методы. Методы органического синтеза, инструментальные методы анализа органических соединений, методы микробиологического скрининга.

Результаты и их обсуждение. Взаимодействием арилсульфонилацетонитрилов с изотиоцианатами в присутствии NaOH и CH_3I и последующей циклизацией с гидразин-гидратом получены 5-амино-4-арилсульфонил-3-фениламинопиразолы. Реакцией этих веществ с *N*-арилхлорацетамидами синтезированы N^1-замещенные 5-амино-4-арилсульфонил-3-фениламинопиразолы. Изучены противомикробные и противогрибковые свойства полученных веществ; некоторые из них являются потенциальными ингибиторами патогенных бактерий и грибов.

Выводы. Предложена синтетическая схема получения N^1-замещенных 5-амино-4-арилсульфонил-3-фениламинопиразолов, которая может быть применена для создания библиотеки веществ для противомикробного скрининга *in vitro*. Некоторые из синтезированных соединений представляют определенный интерес как потенциальные фармацевтические агенты и могут быть использованы для разработки новых противогрибковых агентов.

***Ключевые слова*:** *5-аминопиразол; синтез; противомикробная активность; противогрибковая активность*

Various substituted aminopyrazoles are an important scaffold for medical chemistry and present in the core of many pharmacological agents. For example, Ceftolozane is a cephalosporin antibiotic of the 5th generation [1], Tozasertib is a pan-Aurora inhibitor [2]. Polysubstituted derivatives of pyrazole can act as antibacterial [3-6], anti-inflammatory [3, 7, 8], cytostatic [2, 9, 10], anesthetizing [11], anticancer [12-15], antiepileptic agents [16-18] and as insecticides [19, 20].

These examples illustrate the ongoing interest toward new small molecules with the pyrazole ring and have prompted us to explore the synthetic route of chemical modification of 3,5-diaminopirazole, which can serve as a promising source of bioactive molecules.

Experimental Part

All reagents and solvents were obtained from the commercial sources. Elemental analysis was performed on a Euro EA-3000 apparatus. Melting points were obtained by a Buchi B-520 device. The NMR-spectra were recorded with a Bruker 170 Avance spectrometer at 200 MHz, 500 MHz (DMSO-d_6); TMS was used as an internal standard; chemical shifts were reported in ppm. LC/MS spectra were recorded with a PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (215 and 254 nm) and using a C18 column (100 × 4 mm). Elution started with water and ended with acetonitrile/water (95 : 5, v/v); a linear gradient at the flow rate of 0.15 mL/min and the cycle time of 25 min was used. According to LC/MS data all compounds synthesized had purity > 95 %. The TLC was performed on the aluminum plates covered with a silica gel (Merck, Kiesgel 60 F-254).

The general procedure for 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles (1.1-1.2). To the solution of sodium hydroxide (85 mmol, 3.4 g) in water (100 mL) add the mixture of the corresponding arylsulfonylacetonitrile (80 mmol) and phenylisothiocyanate (85 mmol, 11.47 g) in dioxane (150 mL) and stir at room temperature. After that add methyl iodide (85 mmol, 5.3 g), and stir for 3 h at room temperature. Dilute the reaction mixture with cool water (200 mL). Filter the precipitate formed and wash with water and propanole-2 – water mixture (1 : 1).

Add hydrazine hydrate (66 mmol, 3.3 mL) and few drops of triethylamine to the corresponding *N,S*-acetale (60 mmol) in propanole-2 (180 mL) and reflux for 3 h. After that dilute the solution with water (150 mL). Filter the precipitate formed and wash with water and propanole-2 – water mixture (1 : 1).

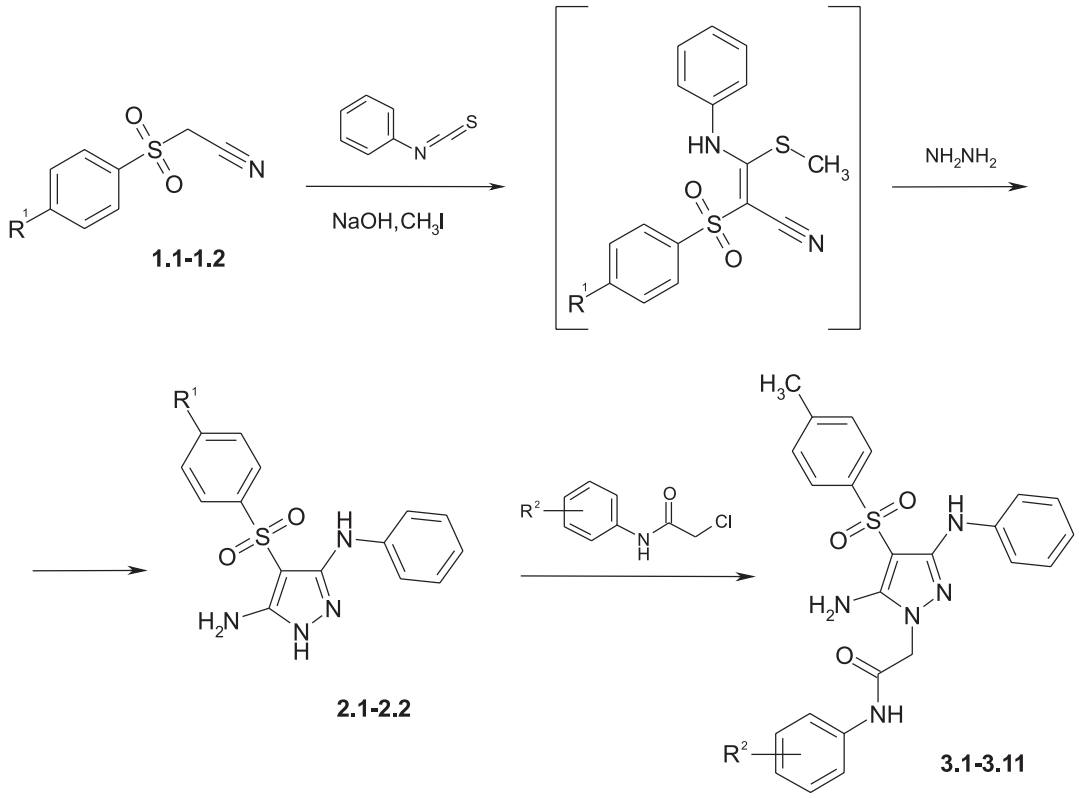
5-Amino-4-phenylsulfonyl-3-phenylaminopyrazole **1.1**. Yield – 91 %. M. p. – 196 °C. ^{1}H NMR δ: 6.10 (s, 2H, NH_2), 6.81 (t, 1H, NH), 7.18 (t, 2H, Ar-H), 7.71 (m, 6H, Ar-H), 7.95 (d, 2H, Ar-H), 11.30 (s, 1H, NH).

5-Amino-4-(4'-methyl)phenylsulfonyl-3-N-phenylaminopyrazole **1.2**. Yield – 96 %. M. p. – 206-07 °C. ^{1}H NMR δ: 2.29 (s, 3H, CH_3), 6.08 (s, 2H, NH_2), 6.75 (t, 1H, NH), 7.21 (t, 2H, Ar-H), 7.33 (d, 2H, Ar-H), 7.49 (m, 3H, Ar-H), 7.86 (d, 2H, Ar-H), 11.27 (s, 1H, NH).

The general procedure for N^1-alkylation 5-amino-4-(4'-metylphenyl)sulfonyl-3-*N*-phenylaminopyrazole. To the mixture of 5-amino-4-(4'-methylphenyl)sulfonyl-3-*N*-phenylaminopyrazole (5 mmol, 1.64 g) and K_2CO_3 (15 mmol, 0.87 g) in DMF (50 mL) add the appropriate *N*-arylchloroacetamide (6 mmol). Stir the reaction mixture at 80 °C for 1 h, then cooled to room temperature. Add water (150 mL), filter the precipitate formed, and crystallize from the mixture of ethanol – DMF (1 : 1).

Microbiological experiment

The antibacterial and antifungal activity was determined *in vitro* by the double dilution method in the Anti bacterial Agents Laboratory of Mechnikov Institute of Microbiology and Immunology. As a microbial model a set of clinical and reference strains of microorganisms such as *Escherichia coli* ATCC 25922 (F-50), *Staphylococcus aureus* ATCC 25923 (F-49), *Bacillis anthracoides*



1.1, **2.1** R^1 = H; **1.2**, **2.2** R^1 = CH_3; **3.1** R^2 = H; **3.2** R^2 = 2-F; **3.3** R^2 = 2,4-diF; **3.4** R^2 = 2-Cl-4-F; **3.5** R^2 = 2-CH_3-5-F; **3.6** R^2 = 4-*i*-Pr; **3.7** R^2 = 2,4-diCH_3; **3.8** R^2 = 3,5-diCH_3; **3.9** R^2 = 3-OCH_3; **3.10** R^2 = 4-OC_2H_5; **3.11** R^2 = 3,4-diOCH_3

Scheme. Preparation of *N*^{1}-substituted 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazoles

ATCC 1312, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans ATCC 885-653* were used. As a standard of the antibacterial action Palinum and Nevigramon were chosen due to their significant activity against most gram-negative and gram-positive bacteria. Moreover, they are widely used in antimicrobial therapy. As a standard of the antifungal action Fluconazolum was used.

Results and discussion

A convenient and effective synthetic scheme for preparation *N*^{1}-subsituted 4-alkyl/arylsulfonyl-5-amino-3-alkylthiopyrazoles and their acylation products [5] was developed. The key procedures, which led to the target compounds, were the synthesis of the corresponding *S,S*-acetales and their cyclization with hydrazine hydrate. In this paper, using the similar synthetic scheme, a new series of 4-arylsulfonylderivatives of 5-aminopyrazoles with 3-arylamino fragment was obtained. For this purpose arylisothiocyanates were used at the first stage of interaction, and as intermediates *N,S*-acetales were prepared. The reaction of arylsulfonylacetonitriles **1.1**-**1.2** with phenylisothiocyanate in the presence of NaOH with further addition of CH_3I led to *N,S*-acetales with the yields over 90 %. Then *N,S*-acetales obtained were treated with hydrazine hydrate in the boiling propanole-2. The resulting products **2.1**-**2.2** precipitated from the reaction mixture. This approach allows forming amino substitutes in position 3 of pyrasole even before the stage of cyclisation (Scheme).

The reaction of 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazole **2.2** with several substituted *N*-arylchloroacetamides in dimethylformamide (DMF) in the presence of K_2CO_3 at 80 °C led to *N*^{1}-substituted pyrazoles **3.1-3.11**. The yields of the target products depended on the nature of substitutes in aryl fragments. Recrystallization from propanole-2 resulted in **3.1-3.11** as light yellow residues with 61-88 % yields (Tab. 1).

Both elemental and spectral analysis data of all compounds obtained are in agreement with the suggested molecular structures. Thus, the absence of signals of NH protons of ^{1}H NMR-spectra of target products **3.1-3.11** indicates the reaction of *N*^{1}-alkylation. This direction of the reaction was also proven by the NOESY experiment for target compounds. The cross-coupling between the CH_2 protons of the acetamide fragment (near 4.80 ppm) and the amino group (near 6.50 ppm) was observed.

^{1}H NMR-spectra of compounds **3.1-3.11** also showed a characteristic signal of NH_2 protons at δ 6.40…6.47 ppm, the NH signal of the arylamide fragment at δ 9.43…10.42 ppm, the singlet signal of the CH_2 group – at 4.60…4.78 ppm; the signal of the NH proton was superimposed with aromatic protons and observed near δ 7.5 ppm.

All compounds synthesized were subjected to preliminary evaluation of their *in vitro* activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherihia coli*, *Proteus vulgaris* and *Bacillis anthracoides* and the antifungal activity against *Candida albicans*.

Tab. 2 shows MIC (minimal inhibitory concentration, mg/mL) values of *N*^{1}-substituted 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazoles **3.1-3.11**. Most compounds analyzed were active *in vitro* experiments. Thus, eight compounds demonstrated a significant activity against one or more strains of gram-negative and

Table 1

N[1]-Substituted 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazoles

Compound	R^2	Yield, %	M. p., °C	^{1}H NMR δ, ppm (DMSO, 200 MHz)
3.1	H	61	278-80	2.30 (s, 3H, CH_3), 4.65 (s, 2H, CH_2), 6.42 (s, 2H, NH_2), 6.79 (t, 1H, Ar-H), 7.02 (t, 1H, Ar-H), 7.17-7.35 (m, 5H, Ar-H), 7.25 (s, 1H, Ar-H), 7.50 (m 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.17 (s, 1H, NH)
3.2	2-F	78	271-72	2.32 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 6.43 (s, 2H, NH_2), 6.80 (t, 1H, Ar-H), 7.18 (m, 4H, Ar-H), 7.34 (d, 2H, Ar-H), 7.51 (s+m, 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.25 (s, 1H, NH)
3.3	2,4-diF	82	264-66	2.30 (s, 3H, CH_3), 4.78 (s, 2H, CH_2), 6.43 (s, 2H, NH_2), 6.80 (t, 1H, Ar-H), 6.92 (m, 1H, Ar-H), 7.20 (m, 2H, Ar-H), 7.36 (m, 3H, Ar-H), 7.48 (s+d, 3H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.20 (s, 1H, NH)
3.4	2-Cl-4-F	70	> 300	2.30 (s, 3H, CH_3), 4.70 (s, 2H, CH_2), 6.40 (s, 2H, NH_2), 6.85 (m, 2H, Ar-H), 7.20-7.35 (m, 6H, Ar-H), 7.50 (s+m, 4H, NH+Ar-H), 7.90 (d, 2H, Ar-H), 10.42 (s, 1H, NH)
3.5	2-Me-5-F	80	291-92	2.12 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 4.70 (s, 2H, CH_2), 6.45 (s, 2H, NH_2), 6.83 (m, 2H, Ar-H), 7.17 (m, 3H, Ar-H), 7.34 (d, 2H, Ar-H), 7.50 (m, 4H, NH+Ar-H), 7.85 (d, 2H, Ar-H), 9.43 (s, 1H, NH)
3.6	4-i-Pr	68	218-19	1.10 (d, 6H, $2CH_3$), 2.29 (s, 3H, CH_3), 2.76 (m, 1H, CH), 4.62 (s, 2H, CH_2), 6.42 (s, 2H, NH_2), 6.81 (t, 1H, Ar-H), 7.16 (m, 4H, Ar-H), 7.35 (d, 2H, Ar-H), 7.48 (m, 5H, NH+Ar-H), 7.89 (d, 2H, Ar-H), 10.05 (s, 1H, NH)
3.7	2,4-diMe	88	269-70	2.11 (s, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 6.43 (s, 2H, NH_2), 6.67 (s, 1H, Ar-H), 6.83 (t, 1H, Ar-H), 7.16 (m, 4H, Ar-H), 7.34 (d, 2H, Ar-H), 7.48 (s+d, 3H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.02 (s, 1H, NH)
3.8	3,5-diMe	81	248	2.11 (s, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 6.47 (s, 2H, NH_2), 6.64 (s, 1H, Ar-H), 7.03 (s, 3H, Ar-H), 7.18 (t, 2H, Ar-H), 7.34 (d, 2H, Ar-H), 7.54 (m, 3H, NH+Ar-H), 7.88 (d, 2H, Ar-H), 9.47 (s, 1H, NH)
3.9	3-OMe	74	231-33	2.28 (s, 3H, CH_3), 3.67 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2), 6.42 (s, 2H, NH_2), 6.61 (dd, 1H, Ar-H), 6.80 (t, 1H, Ar-H), 7.04 (dd, 1H, Ar-H), 7.17 (t, 3H, Ar-H), 7.30 (m, 1H, Ar-H), 7.32 (d, 2H, Ar-H), 7.48 (s+d, 3H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.20 (s, 1H, NH)
3.10	4-OEt	79	> 300	1.13 (t, 3H, $CH_2C\underline{H}_3$), 2.27 (s, 3H, CH_3), 3.90 (q, 2H, $C\underline{H}_2CH_3$), 4.61 (s, 2H, CH_2), 6.42 (s, 2H, NH_2), 6.81 (m, 3H, Ar-H), 7.18 (t, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 7.48 (m, 5H, NH+Ar-H), 7.87 (d, 2H, Ar-H), 10.01 (s, 1H, NH)
3.11	3,4-diOMe	69	256-57	2.30 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 4.60 (s, 2H, CH_2), 6.40 (s, 2H, NH_2), 6.80 (t, 1H, Ar-H), 6.87 (m, 1H, Ar-H), 6.99 (dd, 1H, Ar-H), 7.16 (t 2H, Ar-H), 7.32 (m, 3H, Ar-H), 7.49 (d, 2H, Ar-H), 7.52 (s, 1H, NH), 7.86 (d, 2H, Ar-H), 10.03 (s, 1H, NH)

Table 2

The antibacterial and antifungal activity of *N*[1]-substituted 5-amino-4-(4'-methylphenyl)sulfonyl-3-phenylaminopyrazoles

Compound	*Staphylococcus aureus* ATCC 25923	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 4636	*Bacillis anthracoides* ATCC 1312	*Candida albicans* ATCC 885-653
MIC, mg/mL						
3.1	50	50	50	25	25	25
3.2	50	25	50	100	50	25
3.3	25	50	25	100	100	12,5
3.4	25	25	100	100	100	12,5
3.5	50	50	50	100	100	50
3.6	50	50	100	100	25	50
3.7	25	25	50	50	50	100
3.8	50	25	100	100	100	12,5
3.9	25	50	50	100	100	100
3.10	50	50	100	100	100	12,5
3.11	50	50	100	50	100	50
Palinum	6,25	25	12,5	12,5	12,5	
Nevigramon	50	6,25	50		6,25	
Fluconazolum						50

gram-positive bacteria with MIC value of 25.0 mg/mL. Four compounds were active against *E. coli*; three of them revealed the same efficacy as Palinum (MIC 25 mg/mL), two compounds (**3.4**, **3.7**) exhibited the efficacy inhibiting of *E. coli* and *St. aureus*. However, all compounds synthesized were practically inactive against *Pr. vulgaris and B. anthracoides*.

Most derivatives of pyrazole analyzed were active against *C. albicans*: six of them revealed better efficacy than the standard antifungal drug, Fluconazolum. The most active antifungal compounds were **3.3**, **3.4**, **3.8** and **3.10** (MIC 12.5 mg/mL).

One can assume that synthesized compounds have a broad potential of the pharmacological activity especially as antifungal agents; and development of the methods of synthesis and the study of pharmacological properties of these compounds are topical directions of pharmaceutical and medical chemistry.

CONCLUSIONS

The synthetic scheme for obtaining of N^1-substituted 5-amino-4-arylsulfonyl-3-phenylaminopyrazoles, which can be used for creation of a library of compounds for *in vitro* antimicrobial screening, has been proposed. Some of the compounds synthesized are of certain interest as potential pharmaceutical agents and can be used to develop new antifungal agents.

Conflict of Interests: authors have no conflict of interests to declare.

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