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# Synthesis and Antifungal Properties of Some Nitroimidazole Derivatives

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## Abstract

A series of new nitroimidazole derivatives were synthesized and evaluated as antifungal agents. It was found that some of these products were moderate to good protectors against tested fungi species. The best fungal inhibition was observed for compound having methyl benzoate moiety and 2-methyl group in the imidazole ring. Moreover, some attempts of correlation of biological activity with clogP and PSA values of obtained products were performed.

**Keywords:** nitroimidazoles, p-hydroxybenzoates, nucleophilic substitution, ring opening reaction, antifungal activity

## Contents



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## 1. Introduction

Fungal diseases are still the serious threat to human health [1]. In recent years, the severity of these infections has increased. They are particularly dangerous for immuno-compromised patients [2]. Some strains of *Aspergillus* species cause serious lung disease and fungal ear infections (otomycosis). Important risk to humans is also fungi growing in residential buildings, and particularly important types are *Penicillium* and *Aspergillus* [3]. Mycotoxin produced by fungi can cause allergies, dizziness, fainting and could be mutagenic and carcinogenic [4]. Besides, pathogenic microorganisms have developed the resistance to commonly used drugs, so there is an urgent need to obtain new active compounds. Imidazole and nitroimidazole derivatives have broad spectrum of pharmacological activities [5, 6, 7]. They are used mainly as antifungal, antibacterial and antiprotozoal agents. Many of these compounds can act as radiosensitizers, tuberculostatics and anti-HIV drugs. As antifungal agents, imidazole

derivatives are the inhibitors of the ergosterol synthesis in the cell membrane [2]. These compounds act as blockers of the active site of an enzyme known as lanosterol 14α-demethylase or cytochrome $P450_{DM}$ [8]. Imidazoles inhibit the synthesis of normal membrane sterols in fungi. Lack of ergosterol in a fungal membrane seriously perturbs further growing and development of fungi. One of the most important drug among antifungal azoles is ketoconazole – a compound that includes imidazole moiety. It can be used against the broad spectrum of fungi species, such as: *Candida spp*, *Coccidioides spp*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* [9]. Moreover, many authors have described the synthesis and promising results of antifungal tests for different heterocyclic compounds [10, 11, 12, 13] including imidazole and nitroimidazole derivatives [14, 15]. Mechanism of action of imidazoles and nitroimidazoles for ergosterol, caused the interdisciplinary use of drugs in this group. The literature shows examples of the use of nitroimidazoles in plant protection and preservation of cellulosic materials [15].

The broad spectrum of biological activity of imidazole derivatives was the motivation to synthesize a set of new heterocyclic products. The aim of the research was to obtain new active substances acording to the reactions of nitroimidazodihydrooxazoles (**1**, **2**) with secondary cyclic amines, thiophenols and phenols in different conditions. Moreover, the practical purpose was to use new derivatives of imidazoles to protect wood against microfungi. Additionally, some attempts were taken to correlate biological activity with C log P and PSA values.

High bioavailability is an important factor for the development of bioactive agents. Essential predictors of good drug distribution include passive intestinal absorption, reduced molecular flexibility, proper degree of lipophilicity (log P value), total hydrogen bond count and low polar surface area (PSA) defined as the sum of surface contributions of polar atoms in a molecule [16, 17]. Most active compounds have log P ≤ 5 and PSA ≤ 140 Å. High PSA values (PSA > 250 Å) are correlated with significantly decreased bioavailability as well as distribution possibilities [18]. We have calculated log P and PSA values for all synthesized compounds using molinspiration software programs.

## 2. Materials and Method

Commercially available solvents and chemicals were used without further purification. Melting points were determined in a Kofler's apparatus and are uncorrected. All compounds have sharp melting points. The $^{1}H$ and $^{13}C$ NMR spectra were recorded in DMSO-$d_6$ solutions with use of TMS as an internal standard on a Varian Gemini 300 VT and Mercury 300 spectrometers at 300 ($^{1}H$) and 75 MHz ($^{13}C$). MS spectra were recorded on a 402 AMD INTECTRA apparatus by the electron impact and high resolution techniques, operating at 75 eV. HR MS spectra were recorded for new products that underwent antifungal test. Analytical TLC was performed on Merck silica gel $60F_{254}$ plates using methylene chloride-methanol (9:1 v/v) mixture as eluent. The spots were observed in the UV light (λ = 254 nm).

**General procedure for the synthesis of compounds 3 and 4**

0.84 mmol of appropriate nitroimidazodihydrooxazole **(1)** or **(2)** and 0.84 mmol of thiophenol were mixed in 10 mL of EtOH in room temperature. Then 0.11 g (0.84 mmol) of $K_2CO_3$ was added. The mixture was left for 1 hour, and then 40 mL of cold water was dropped. The yellow precipitate was filtered off, washed with small amount of water, air-dried and crystallized from 25% EtOH. Spectral and analytical data for compounds **3, 4** are as follows.

**3-Chloro-1-(4-nitro-5-thiophenoxyimidazol-1-yl)propan-2-ol (3)**:

0.21 g (82% yield), yellow needles, mp. 92-93°C, $R_f$ = 0.65;

$^{1}H$ NMR: δ 8.13 (s, 1H, Im), 7.19-7.37 (m, 5H, Ph), 5.78 (d, *J* = 5.5 Hz, 1H, OH), 4.28-4.34 (m, 1H, C**H**-OH), 4.07-4.15 (m, 1H, N-$CH_2$), 3.86-3.92 (m, 1H, N-$CH_2$), 3.56-3.67 (m, 2H, $CH_2Cl$);

$^{13}C$ NMR: δ 148.57 (C-4 Im), 139.50 (Ph), 133.37 (Ph), 129.63 (C-2 Im), 127.64 (Ph), 127.20 (Ph), 121.86 (C-5 Im), 68.83 (CH-OH), 49.19 (N-$CH_2$), 46.67 ($CH_2Cl$);

MS *m/z* (%): 313.1 (34.4), 315.1 (17.0) [$M^+$].

**3-Chloro-1-(4-nitro-5-(*p*-chlorothiophenoxy)imidazol-1-yl)propan-2-ol(4)**:

0.27 g (94% yield), yellow needles, mp. 124-126°C, $R_f$ = 0.72;

$^{1}H$ NMR: δ 8.15 (s, 1H, Im), 7.12-7.57 (m, 4H, Ph), 5.80 (d, *J* = 5.5 Hz, 1H, OH), 4.29-4.38 (m, 1H, C**H**-OH), 4.09-4.16 (m, 1H, N-$CH_2$), 3.87-3.92 (m, 1H, N-$CH_2$), 3.58-3.69 (m, 2H, $CH_2Cl$);

$^{13}C$ NMR: δ 148.36 (C-4 Im), 139.38 (Ph), 132.34 (Ph), 129.30 (C-2 Im), 129.17 (Ph), 129.02 (Ph), 121.29 (C-5 Im), 68.83 (CH-OH), 49.18 (N-$CH_2$), 46.60 ($CH_2Cl$);

MS *m/z* (%): 349.0 (19.8), 351.0 (5.7) [$M^+$].

**General procedure for the synthesis of compounds 5 - 8**

To a solution of 0.84 mmol of appropriate nitroimidazodihydrooxazole **(1)** or **(2)** in 10 mL of EtOH, a respective secondary cyclic amine (0.84 mmol): morpholine or piperidine and 0.11 g (0.84 mmol) $K_2CO_3$ were added. The mixture was left in room temperature. After 1 hour, 40 mL of cold water was dropped. The yellow precipitate was

filtered off, washed with small amount of water, air-dried and crystallized from 25% EtOH. Spectral and analytical data for compounds **5 – 8** are as follows.

**3-Chloro-1-(5-morpholine-4-nitroimidazol-1-yl)propan-2-ol (5)**:
0.18 g (76% yield), yellow needles, mp. 124-126°C, $R_f$ = 0.50;
$^1$H NMR: δ 7.68 (s, 1H, Im), 5.75 (d, *J* = 5.3 Hz, 1H, OH), 3.90-4.18 (m, 3H, C**H**-OH, N-$CH_2$), 3.63-3.76 (m, 6H, $CH_2$Cl, 2x$CH_2$ 3,5-morpholine), 2.99-3.18 (m, 4H, 2x$CH_2$ 2,6-morpholine);
$^{13}$C NMR: δ 140.36 (C-4 Im), 138.93 (C-2 Im), 133.34 (C-5 Im), 68.85 (CH-OH), 66.38 (2x$CH_2$ morpholine), 49.18 (N-$CH_2$), 47.27 (2x$CH_2$ morpholine), 46.90 ($CH_2$Cl);
MS *m/z* (%): 290.0 (47.2), 292.0 (26.7) [$M^+$]. HRMS m/z: calcd for $C_{10}H_{15}N_4O_4{}^{35}Cl$ 290.0782, found: 290.0771.

**3-Chloro-1-(2-methyl-5-morpholine-4-nitroimidazol-1-yl)propan-2-ol (6)**:
0.22 g (87% yield), yellow needles, mp. 185-186°C, $R_f$ = 0.53;
$^1$H NMR: δ 5.71 (d, *J* = 4.9 Hz, 1H, OH), 3.91-4.11 (m, 3H, C**H**-OH, N-$CH_2$), 3.67-3.77 (m, 6H, $CH_2$Cl, 2x$CH_2$ 3,5-morpholine), 2.99-3.26 (m, 4H, 2x$CH_2$ 2,6-morpholine), 2.34 (s, 3H, $CH_3$);
$^{13}$C NMR: δ 141.30 (C-4 Im), 139.34 (C-2 Im), 138.51 (C-5 Im), 69.01 (CH-OH), 66.44 (2x$CH_2$ morpholine), 48.45 (N-$CH_2$), 47.08 (2x$CH_2$ morpholine), 46.64 ($CH_2$Cl), 13.95 ($CH_3$);
MS *m/z* (%): 304.0 (73.6), 306.0 (40.1) [$M^+$].

**3-Chloro-1-(4-nitro-5-piperidinoimidazol-1-yl)propan-2-ol (7)**:
0.19 g (80% yield), yellow needles, mp. 127-128°C, $R_f$ = 0.56;
$^1$H NMR: δ 7.62 (s, 1H, Im), 5.76 (d, *J* = 5.1 Hz, 1H, OH), 3.84-4.14 (m, 3H, C**H**-OH, N-$CH_2$), 3.62-3.68 (m, 2H, $CH_2$Cl), 2.94-3.12 (m, 4H, 2x$CH_2$ 2,6-piperidine), 1.56-1.62 (m, 6H, 3x$CH_2$ 3,4,5-piperidine);
$^{13}$C NMR: δ 140.42 (C-4 Im), 138.40 (C-2 Im), 133.14 (C-5 Im), 68.78 (CH-OH), 49.76 (N-$CH_2$), 47.29 (2x$CH_2$ 2,6-piperidine), 46.84 ($CH_2$Cl), 25.71 (2x$CH_2$ 3,5-piperidine), 23.48 ($CH_2$ 4-piperidine);
MS *m/z* (%): 288.0 (21.3), 289.9 (10.5) [$M^+$]. HRMS m/z: calcd for $C_{11}H_{17}N_4O_3{}^{35}Cl$ 288.0989, found: 288.0993.

**3-Chloro-1-(2-methyl-4-nitro-5-piperidinoimidazol-1-yl)propan-2-ol (8)**:
0.21 g (85% yield), yellow needles, mp. 158-159°C, $R_f$ = 0.62;
$^1$H NMR: δ 5.70 (d, *J* = 5.1 Hz, 1H, OH), 3.84-4.06 (m, 3H, C**H**-OH, N-$CH_2$), 3.68-3.71 (m, 2H, $CH_2$Cl), 2.93-4.00 (m, 4H, 2x$CH_2$ 2,6-piperidine), 2.32 (s, 3H, $CH_3$), 1.55-1.63 (m, 6H, 3x$CH_2$ 3,4,5-piperidine);
$^{13}$C NMR: δ 140.87 (C-4 Im), 140.61 (C-2 Im), 137.90 (C-5 Im), 69.05 (CH-OH), 49.50 (N-$CH_2$), 46.96 (2x$CH_2$ 2,6-piperidine), 46.68 ($CH_2$Cl), 25.67 (2x$CH_2$ 3,5-piperidine), 23.51 ($CH_2$ 4-piperidine), 14.00 ($CH_3$);
MS *m/z* (%): 302.2 (27.5), 304.3 (32.1) [$M^+$]. HRMS m/z: calcd for $C_{12}H_{19}N_4O_3{}^{35}Cl$ 302.1146, found: 302.1143.

**General procedure for the synthesis of compounds 9 – 12:**
To a solution of 0.84 mmol of appropriate nitroimidazodihydrooxazole **(1)** or **(2)** in 10 mL of EtOH, a respective thiophenol (3.36 mmol) and 0.44 g (0.84 mmol) $K_2CO_3$ was added. The mixture was heated under reflux. After 1 hour, 40 mL of cold water was dropped. The yellow precipitate was filtered off, washed with small amount of water, air-dried and crystallized from water. Spectral and analytical data for compounds **9 – 12** are as follows.

**3-Thiophenoxy-1-(4-nitro-5-thiophenoxyimidazol-1-yl)propan-2-ol (9)**:
0.32 g (100% yield), yellow needles, mp. 59-61°C, $R_f$ = 0.83;
$^1$H NMR: δ 8.10 (s, 1H, Im), 7.12-7.55 (m, 10H, 2xAr), 5.69 (d, *J* = 5.5 Hz, 1H, OH), 4.35-4.40 (m, 1H, C**H**-OH), 4.04-4.14 (m, 1H, N-$CH_2$), 3.79-3.85 (m, 1H, N-$CH_2$), 3.04-3.18 (m, 2H, $CH_2$-S);
$^{13}$C NMR: δ 148.43 (C-4 Im), 139.45 (Ar), 135.73 (Ar), 133.21 (Ar), 129.47 (C-2 Im), 129.36 (Ar), 128.91 (Ar), 127.27 (Ar), 126.96 (Ar), 125.74 (Ar), 121.39 (C-5 Im), 68.09 (CH-OH), 50.70 (N-$CH_2$), 48.62 ($CH_2$-S);
MS *m/z* (%): 387.0 $M^+$ (7.5).

**3-Thiophenoxy-1-(2-methyl-4-nitro-5-thiophenoxyimidazol-1-yl)propan-2-ol (10)**:
0.34 g (100% yield), yellow needles, mp. 51-53°C, $R_f$ = 0.86;
$^1$H NMR: δ 7.09-7.38 (m, 10H, 2xPh), 5.63 (d, *J* = 5.3 Hz, 1H, OH), 4.29-4.34 (m, 1H, C**H**-OH), 4.03-4.11 (m, 1H, N-$CH_2$), 3.78-3.86 (m, 1H, N-$CH_2$), 3.11-3.13 (m, 2H, $CH_2$-S), 2.46 (s, 3H, $CH_3$);
$^{13}$C NMR: δ 147.57 (C-4 Im), 147.49 (Ar), 135.66 (Ar), 133.62 (Ar), 129.42 (C-2 Im), 128.92 (Ar), 128.19 (Ar), 126.89 (Ar), 126.75 (Ar), 125.76 (Ar), 120.98 (C-5 Im), 68.63 (CH-OH), 49.83 (N-$CH_2$), 37.03 ($CH_2$-S), 14.27 ($CH_3$);
MS *m/z* (%): 401.2 $M^+$ (8.9).

**3-(*p*-Chlorothiophenoxy)-1-(4-nitro-5-(p-chlorothiophenoxy)imidazol-1-yl)propan-2-ol (11)**:
0.38 g (100% yield), yellow needles, mp. 65-67°C, $R_f$ = 0.64;
$^1$H NMR: δ 8.12 (s, 1H, Im), 7.11-7.57 (m, 8H, 2xPh), 5.71 (d, *J* = 5.3 Hz, 1H, OH), 4.36-4.42 (m, 1H, C**H**-OH), 4.03-4.11 (m, 1H, N-$CH_2$), 3.63-3.76 (m, 1H, N-$CH_2$), 3.04-3.11 (m, 2H, $CH_2$-S);
$^{13}$C NMR: δ 148.70 (C-4 Im), 139.78 (Ar), 134.94 (Ar), 134.53 (Ar), 132.57 (Ar), 131.70 (Ar), 130.46 (Ar), 129.77 (Ar), 129.52 (C-2 Im), 128.84 (Ar), 120.91 (C-5 Im), 68.05 (CH-OH), 50.76 (N-$CH_2$), 36.82 ($CH_2$-S);
MS *m/z* (%): 459.1 $M^+$ (2.2).

**3-(*p*-Chlorothiophenoxy)-1-(2-methyl-4-nitro-5-(*p*-chlorothiophenoxy)imidazol-1-yl)propan-2-ol (12)**:
0.39 g (100% yield), yellow needles, mp. 95-96°C, $R_f$ = 0.72;

$^1$H NMR: δ 7.08-7.35 (m, 8H, 2xPh), 5.65 (d, *J* = 5.3 Hz, 1H, OH), 4.29-4.35 (m, 1H, C**H**-OH), 4.02-4.10 (m, 1H, N-$CH_2$), 3.73-3.77 (m, 1H, N-$CH_2$), 3.07-3.15 (m, 2H, $CH_2$-S), 2.47 (s, 3H, $CH_3$);
$^{13}$C NMR: δ 147.76 (C-4 Im), 147.54 (Ar), 134.69 (Ar), 132.79 (Ar), 131.30 (Ar), 130.28 (Ar), 129.53 (Ar), 129.21 (Ar), 128.73 (C-2 Im), 128.29 (Ar), 120.32 (C-5 Im), 68.53 (CH-OH), 49.87 (N-$CH_2$), 38.67 ($CH_2$-S), 14.29 ($CH_3$);
MS *m/z* (%): 475.4 $M^+$ (1.4).

**General procedure for the synthesis of compounds 13 – 16:**
To a solution of 0.84 mmol of appropriate nitroimidazodihydrooxazole **(1)** or **(2)** in 10 mL of EtOH, a respective secondary cyclic amine (3.36 mmol): morpholine or piperidine and 0.44 g (3.36 mmol) $K_2CO_3$ was added. The mixture was heated under reflux. After 1 hour, 40 mL of cold water was dropped. The yellow precipitate was filtered off, washed with small amount of water, air-dried and crystallized from water. If there was no precipitate, mixture was extracted with $CH_2Cl_2$ and after evaporation of organic solvent, product was isolated by silica gel column chromatography with $CH_2Cl_2$:$CH_3OH$ (9:1, v/v) as developing solvent. Spectral and analytical data for compounds **13 – 16** are as follows.

**3-Morpholino-1-(5-morpholino-4-nitroimidazol-1-yl)propan-2-ol (13)**:
0.24 g (86% yield), yellow needles, mp. 110-112°C, $R_f$ = 0.34;
$^1$H NMR: δ 7.66 (s, 1H, Im), 5.16 (d, *J* = 5.1 Hz, 1H, OH), 4.13-4.18 (m, 1H, C**H**-OH), 3.57-4.08 (m, 8H, N-$CH_2$, $CH_2$-N, 2x$CH_2$ morpholine), 3.00-3.17 (m, 4H, 2x$CH_2$ morpholine), 2.35-2.52 (m, 8H, 4x$CH_2$ morpholine);
$^{13}$C NMR: δ 138.98 (C-4 Im), 138.82 (C-2 Im), 133.45 (C-5 Im), 66.49 (CH-OH), 66.31 (2x$CH_2$ morpholine), 66.20 (2x$CH_2$ morpholine), 62.47 (2x$CH_2$ morpholine), 54.00 (2x$CH_2$ morpholine), 48.72 (N-$CH_2$), 48.56 ($CH_2$-N);
MS *m/z* (%): 341.3 $M^+$ (1.0).

**3-Morpholino-1-(2-methyl-5-morpholino-4-nitroimidazol-1-yl)propan-2-ol (14):**
0.16 g (54% yield), yellow needles, mp. 152-154°C, $R_f$ = 0.37;
$^1$H NMR: δ 5.12 (d, *J* = 4.7 Hz, 1H, OH), 4.03-4.08 (m, 1H, C**H**-OH), 3.49-3.89 (m, 8H, N-$CH_2$, $CH_2$-N, 2x$CH_2$ morpholine), 3.00-3.34 (m, 4H, 2x$CH_2$ morpholine), 2.36-2.55 (m, 8H, 4x$CH_2$ morpholine), 2.33 (s, 3H, $CH_3$);
$^{13}$C NMR: δ 141.13 (C-4 Im), 139.17 (C-2 Im), 138.24 (C-5 Im), 66.63 (CH-OH), 66.48 (2x$CH_2$ morpholine), 66.22 (2x$CH_2$ morpholine), 62.76 (2x$CH_2$ morpholine), 54.12 (2x$CH_2$ morpholine), 48.38 (N-$CH_2$), 48.29 ($CH_2$-N), 14.06 ($CH_3$);
MS *m/z* (%): 355.1 $M^+$ (1.6).

**3-Piperidino-1-(4-nitro-5-piperidinoimidazol-1-yl)propan-2-ol (15)**:
0.14 g (50% yield), yellow needles, mp. 58-60°C, $R_f$ = 0.48;
$^1$H NMR: δ 7.58 (s, 1H, Im), 5.10 (d, *J* = 4.5 Hz, 1H, OH), 4.10-4.16 (m, 1H, C**H**-OH), 3.71-3.84 (m, 1H, N-$CH_2$), 3.64-3.68 (m, 1H, N-$CH_2$), 3.50-3.54 (m,2H, $CH_2$-N), 2.94-3.12 (m, 4H, 2x$CH_2$ piperidine), 2.24-2.51 (m, 6H, 3x$CH_2$ piperidine), 1.38-1.62 (m, 10H, 5x$CH_2$ piperidine);
$^{13}$C NMR: δ 140.27 (C-4 Im), 138.23 (C-2 Im), 133.30 (C-5 Im), 66.37 (CH-OH), 63.14 (2x$CH_2$ piperidine), 54.85 (2x$CH_2$ piperidine), 49.72 ($CH_2$-N), 49.02 (N-$CH_2$), 25.73 (2x$CH_2$ piperidine), 23.93 (2x$CH_2$ piperidine), 23.43 (2x$CH_2$ piperidine);
MS *m/z* (%): 337.0 $M^+$ (0.6).

**3-Piperidino-1-(2-methyl-4-nitro-5-piperidinoimidazol-1-yl)propan-2-ol (16)**:
0.21 g (72% yield), yellow needles, mp. 129-130°C, $R_f$ = 0.54;
$^1$H NMR: δ 5.06 (d, *J* = 4.5 Hz, 1H, OH), 4.00-4.06 (m, 1H, C**H**-OH), 3.82-3.84 (m, 1H, N-$CH_2$), 3.57-3.68 (m, 1H, N-$CH_2$), 3.50-3.54 (m,2H, $CH_2$-N), 2.94-3.34 (m, 4H, 2x$CH_2$ piperidine), 2.24-2.51 (m, 6+3H, 3x$CH_2$ piperidine, $CH_3$), 1.39-1.60 (m, 10H, 5x$CH_2$ piperidine);
$^{13}$C NMR: δ 141.06 (C-4 Im), 140.56 (C-2 Im), 137.76 (C-5 Im), 66.87 (CH-OH), 63.49 (2x$CH_2$ piperidine), 54.98 (2x$CH_2$ piperidine), 49.48 ($CH_2$-N), 48.61 (N-$CH_2$), 25.71 (2x$CH_2$ piperidine), 23.95 (2x$CH_2$ piperidine), 23.46 (2x$CH_2$ piperidine), 14.09 ($CH_3$);
MS *m/z* (%): 351.1 $M^+$ (0.7). HRMS m/z: calcd for $C_{17}H_{29}N_5O_3$ 351.2270, found: 351.2251.

**General procedure for the preparation of compounds 17-23:**
Compound **2** (0.18 mmol) and the corresponding phenol (0.36 mmol) were dissolved in respective alcohol. Potassium carbonate (0.36 mmol) was then added and the mixture was heated under reflux for 1h. After cooling, 40 mL of cold water was added. The precipitate was filtered off and washed with cold water to give the crude product, which was recrystallized from 25% EtOH with charcoal to afford pure compound as a white solid.
Spectral and analytical data for compounds **17 – 23** are as follows.

**3-(*p*-Acetylphenoxy)-1-(5-ethoxy-2-methyl-4-nitroimidazol-1-yl)propan-2-ol (17)**:
Reaction was performed in ethanol. 0.15 g (51% yield), white needles, mp. 128-130°C, $R_f$ = 0.70;
$^1$H NMR: δ 7.91 (d, *J* = 8.8 Hz, 2H, 2,6-Ph), 7.06 (d, *J* = 9.1Hz, 2H, 3,5-Ph), 5.73 (d, *J* = 4.7 Hz, 1H, OH), 4.38 (q, *J* = 7.0, 2H, at OC**H**$_2$CH$_3$ C-5 Im), 3.91-4.14 (m, 5H, $CH_2CHCH_2$), 2.52 (s, 3H, $COCH_3$), 2.32 (s, 3H, $CH_3$), 1.35 (t, *J* = 7.0 Hz, 3H, $OCH_2$C**H**$_3$ at C-5 Im).

$^{13}$C NMR: δ 195.41 (C=O), 162.16 (Ar), 145.61 (C-4 Im), 132.60 (C-2 Im), 132.06 (Ar), 131.50 (Ar), 130.12 (C-5 Im), 114.32 (Ar), 72.50 ($CH_2$-OAr), 69.68 (CH-OH), 67.03 (N-$CH_2$), 46.78 (O**C**$H_2CH_3$ at C-5 Im), 25.90 (CO**C**$H_3$), 15.13 ($OCH_2$**C**$H_3$ at C-5 Im), 13.0 ($CH_3$ at C-2 Im).
MS *m/z* (%): 363.1 $M^+$ (34.8). HRMS m/z: calcd for $C_{17}H_{21}N_3O_6$ 363.1430, found: 363.1417.

**Methyl 4-(2-hydroxy-3-(5-methoxy-2-methyl-4-nitroimidazol-1-yl)propoxy)benzoate (18)**:
Reaction was performed in methanol. 0.06 g (27% yield), white needles, mp. 158-160°C, $R_f$ = 0.69;
$^1$H NMR: δ 7.93 (d, *J* = 9.1 Hz, 2H, 2,6-Ph), 7.08 (d, *J* = 9.1 Hz, 2H, 3,5-Ph), 5.72 (d, *J* = 5.1 Hz, 1H, OH), 3.94-4.17 (m, 5H, $CH_2CHCH_2$), 4.09 (s, 3H, $OCH_3$ at C-5 Im), 3.82 (s, 3H, $COOCH_3$), 2.32 (s, 3H, $CH_3$ at C-2 Im).
$^{13}$C NMR: δ 165.93 (C=O), 162.21 (Ar), 145.81 (C-4 Im), 131.51 (C-2 Im), 131.30 (Ar), 130.81 (Ar), 122.16 (C-5 Im), 114.61 (Ar), 69.81 (CH-OH), 67.14 (N-$CH_2$), 63.23 ($CH_2$-OAr), 51.91 (COO**C**$H_3$), 46.78 ($OCH_3$ at C-5 Im), 12.99 ($CH_3$ at C-2 Im).
MS *m/z* (%): 365.1 $M^+$ (50.0). HRMS m/z: calcd for $C_{16}H_{19}N_3O_7$ 365.1223, found: 365.1234.

**Methyl 4-(2-hydroxy-3-(5-ethoxy-2-methyl-4-nitroimidazol-1-yl)propoxy)benzoate (19)**:
Reaction was performed in ethanol. 0.08 g (27% yield), white needles, mp. 74-75°C, $R_f$ = 0.69;
$^1$H NMR: δ 7.94 (d, *J* = 9.1 Hz, 2H, 2,6-Ph), 7.06 (d, *J* = 9.1 Hz, 2H, 3,5-Ph), 5.67 (d, *J* = 5.2 Hz, 1H, OH), 4.34 (q, *J* = 7.0 Hz, 2H, OC**H**$_2CH_3$ at C-5 Im), 3.92-4.12 (m, 5H, $CH_2CHCH_2$), 3.82 (s, 3H, $COOCH_3$), 2.33 (s, 3H, $CH_3$ at C-2 Im), 1.36 (t, *J* = 7.0 Hz, 3H, $OCH_2$C**H**$_3$ at C-5 Im).
$^{13}$C NMR: δ 165.80 (C=O), 163.56 (Ar), 146.81 (C-4 Im), 131.66 (C-2 Im), 131.26 (Ar), 128.53 (Ar), 122.13 (C-5 Im), 115.13 (Ar), 70.23 ($CH_2$-OAr), 67.15 (CH-OH), 66.90 (N-$CH_2$), 51.90 (COO**C**$H_3$), 46.69 (O**C**$H_2CH_3$), 15.13 ($OCH_2$**C**$H_3$), 13.03 ($CH_3$ at C-2 Im).
MS *m/z* (%): 379.2 $M^+$ (17.0).

**Methyl 4-(2-hydroxy-3-(2-methyl-4-nitro-5-*n*-propoxy-imidazol-1-yl)propoxy)benzoate (20)**:
Reaction was performed in *n*-propanol. 0.15 g (46% yield), white needles, mp. 125-126°C, $R_f$ = 0.67;
$^1$H NMR: δ 7.93 (d, *J* = 9.1 Hz, 2H, 2,6-Ph), 7.07 (d, *J* = 9.1 Hz, 2H, 3,5-Ph), 5.72 (d, *J* = 4.7 Hz, 1H, OH), 4.26 (t, *J* = 6.6 Hz, 2H, OC**H**$_2CH_2CH_3$ at C-5 Im), 3.93-4.22 (m, 5H, $CH_2CHCH_2$), 3.82 (s, 3H, $COOCH_3$), 2.31 (s, 3H, $CH_3$), 1.73 (m, 2H, $OCH_2$C**H**$_2CH_3$ at C-5 Im), 0.96 (t, *J* = 7.1 Hz, 3H, $OCH_2CH_2$C**H**$_3$ at C-5 Im).
$^{13}$C NMR: δ 165.91 (C=O), 162.18 (Ar), 145.09 (C-4 Im), 131.66 (C-2 Im), 131.30 (Ar), 130.83 (Ar), 122.19 (C-5 Im), 114.56 (Ar), 69.69 ($CH_2$-OAr), 67.09 (CH-OH), 65.88 (N-$CH_2$), 51.96 (COO**C**$H_3$), 47.11 (O**C**$H_2CH_2CH_3$ at C-5 Im), 22.65 ($OCH_2$**C**$H_2CH_3$ at C-5 Im), 12.98 ($CH_3$ at C-2), 10.05 ($OCH_2CH_2$**C**$H_3$ at C-5 Im).
MS *m/z* (%): 393.2 $M^+$ (6.8).

**Propyl 4-(2-hydroxy-3-(2-methyl-4-nitro-5-*n*-propoxy-imidazol-1-yl)propoxy)benzoate (21)**:
Reaction was performed in *n*-propanol. 0.24 g (70% yield), white needles, mp. 72-74°C, $R_f$ = 0.83;
$^1$H NMR: δ 7.94 (d, *J* = 9.1 Hz, 2H, 2,6-Ph), 7.07 (d, *J* = 9.1 Hz, 2H, 3,5-Ph), 5.69 (d, *J* = 4.4 Hz, 1H, OH), 3.89-4.31 (m, 9H, OC**H**$_2CH_2CH_3$ at C-5 Im; OC**H**$_2CH_2CH_3$ Ester, $CH_2CHCH_2$), 2.33 (s, 3H, $CH_3$ at C-2), 1.66-1.78 (m, 4H, $OCH_2$C**H**$_2CH_3$ at C-5 Im; $OCH_2$C**H**$_2CH_3$ Ester), 0.94-0.99 (m, 6H, $OCH_2CH_2$C**H**$_3$ at C-5 Im; $OCH_2CH_2$C**H**$_3$ Ester).
$^{13}$C NMR: δ 165.46 (C=O), 162.11 (Ar), 145.30 (C-4 Im), 138.50 (C-2 Im), 131.28 (Ar), 130.43 (Ar), 122.43 (C-5 Im), 114.52 (Ar), 77.49 ($CH_2$-OAr), 69.74 (CH-OH), 67.09 (O**C**$H_2CH_2CH_3$ Ester), 65.84 (N-$CH_2$), 46.21 (O**C**$H_2CH_2CH_3$ at C-5 Im), 22.64, 21.71 ($OCH_2$**C**$H_2CH_3$ at C-5 Im and $OCH_2$**C**$H_2CH_3$ Ester), 13.69 ($CH_3$ at C-2 Im), 10.42, 10.09 ($OCH_2CH_2$**C**$H_3$ at C-5 Im and $OCH_2CH_2$**C**$H_3$ Ester).
MS *m/z* (%): 421.1 $M^+$ (3.7). HRMS m/z: calcd for $C_{20}H_{27}N_3O_7$ 421.1849, found: 421.1841.

**3-(5-Chloro-2-(2,4-dichlorophenoxy)phenoxy)-1-(5-methoxy-2-methyl-4-nitro-imidazol-1-yl)propan-2-ol (22)**:
Reaction was performed in methanol. 0.21 g (52% yield), white needles, mp. 138-140°C, $R_f$ = 0.84;
$^1$H NMR: δ 6.71-7.73 (m, 6H, Ar), 5.56 (d, *J* = 4.8 Hz, 1H, OH), 3.60-4.13 (m, 8H, $CH_2CHCH_2$, -$OCH_3$ at C-5 Im), 2.18 (s, 3H, $CH_3$ at C-2 Im).
$^{13}$C NMR: δ 152.04 (Ar), 150.55 (Ar), 146.09 (C-4 Im), 141.99 (Ar), 138.16 (C-2 Im), 130.31 (Ar), 130.03 (Ar), 129.89 (Ar), 128.46 (Ar), 126.79 (Ar), 122.96 (Ar), 122.80 (C-5 Im), 121.55 (Ar), 117.82 (Ar), 115.24 (Ar), 70.62 ($CH_2$-OAr), 66.90 (CH-OH), 62.99 (N-$CH_2$), 45.92 ($OCH_3$ at C-5 Im), 13.40 ($CH_3$ at C-2 Im).
MS *m/z* (%): 501.0 $M^+$ (14.6). HRMS m/z: calcd for $C_{20}H_{18}N_3O_6{}^{35}Cl_3$ 501.0261, found: 501.0259.

**3-(4-Allyl-2-methoxyphenoxy)-1-(5-ethoxy-2-methyl-4-nitroimidazol-1-yl)-propan-2-ol (23)**:
Reaction was performed in ethanol. 0.16 g (49% yield), white needles, mp. 85-86°C, $R_f$ = 0.66;
$^1$H NMR: δ 6.92, 6.81, 6.69 (m, 3 x 1H, Ar), 5.95 (m, 1H, $CH_2$C**H**=$CH_2$), 5.60 (d, *J* = 5.2 Hz, 1H, OH), 5.05 (m, 2H, $CH_2CH$=C**H**$_2$), 4.37 (q, *J* = 7.0, 2H, OC**H**$_2CH_3$ at C-5 Im), 4.18-3.85 (m, 5H, $CH_2CHCH_2$), 3.75 (s, 3H, $OCH_3$), 3.33 (m, 2H, C**H**$_2CH$=$CH_2$), 1.35 (t, *J* = 7.1 Hz, 3H, $OCH_2$C**H**$_3$ at C-5 Im), 2.18 (s, 3H, $CH_3$ at C-2 Im).
$^{13}$C NMR: δ 149.09 (Ar), 146.21 (Ar), 145.63 (C-4 Im), 138.55 ($CH_2$**C**H=$CH_2$), 133.04 (C-2 Im), 131.69 (Ar), 130.76 (Ar), 120.65 (C-5 Im), 115.78 ($CH_2CH$=**C**$H_2$), 114.68 (Ar), 112.31 (Ar), 72.50 ($CH_2$-OAr), 70.57 (CH-OH), 67.21 (**C**$H_2CH$=$CH_2$), 67.15 (N-$CH_2$), 55.48 (-$CH_3$), 46.90 (O**C**$H_2CH_3$ at C-5 Im), 15.03 ($OCH_2$**C**$H_3$ at C-5 Im), 13.22 ($CH_3$ at C-2 Im).
MS *m/z* (%): 391.2 $M^+$ (93.3).

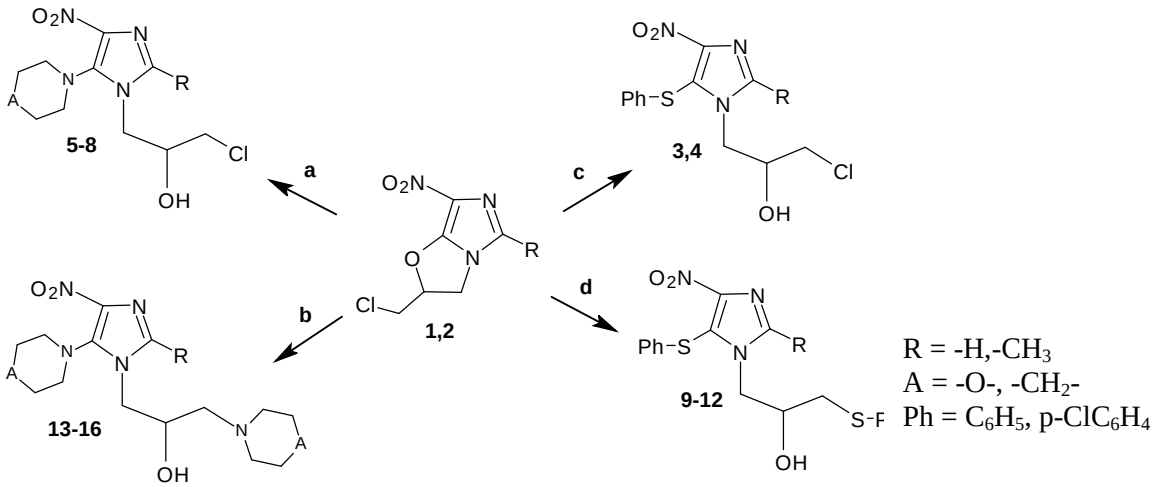
***Biological assays***

The selected synthesized compounds (**5, 7, 8, 16-18, 21, 22, 24, 25**) were tested for their antifungal activity. Working solutions were applied on the nutrient medium - Whatmann filter paper containing 97% α-cellulose. Samples of the filter paper treated with working solution through the soaking method for 60s at the application rate between 165 and 185g/m$^2$ were exposed on malt agar with Czapek-Dox salt. The antifungal efficacy of the selected compounds was performed against the following microfungi species: *Aspergillus niger* Van Tieghem (An), *Trichoderma viride* Pers. ex. S.F. Gray aggr. (Tv), *Penicillium funiculosum* Thom. (Pf), *Paecilomyces variotti* Bainier (Pv), Thom. and their mixtures. The purpose of use of microfungi mixture was mapping of naturally occurring biological agent. The growth rate was visually determined acc. to modified ASTM D 5590-94 (Table 4) after 4, 7, 14 and 21 days. After 4 days untreated samples were totally overgrown by fungi. Determination after 4, 7, 14 and 21 days shows the dynamics of microfungi growth rate.

## 3. Results and Discussion

**Chemistry**

In our earlier works [19, 20], substitution of the halogen atom in the –$CH_2X$ group in 2-chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole system with phenols [19] and primary amines [20] has been described. The main feature of these syntheses is dihydrooxazole ring opening reaction. This mechanism has been used for forming new nitroimidazole derivatives with thiophenol and secondary amine moieties as a result of nucleophilic substitution reaction in 2-chloromethyl-7-nitroimidazo [5,1-*b*]-2,3-dihydrooxazole (**1**) and 2-chloromethyl-5-methyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**2**). Treatment of the nitroimidazodihydrooxazole with equimolar amount of amine or thiophenol furnished the products with one amino or thiopheno group substituted at C-5 position of nitroimidazole ring. Increasing this ratio to 4 equivalents of nucleophile lead to form respective derivatives with two newly introduced cyclic moieties – the first one at C-5 position and the second one – at N-1 alkyl chain, as a result of nucleophilic substitution of chlorine atom.




**Scheme 1:** Synthesis of amino- and thiopheno-nitroimidazole derivatives

**Reactions conditions:** a = **1** or **2**, morpholine or piperidine, $K_2CO_3$ (1:1:1), EtOH; b = **1** or **2**, morpholine or piperidine, $K_2CO_3$ (1:4:4), EtOH; c = **1**, thiophenol or p-chlorothiophenol, $K_2CO_3$ (1:1:1), EtOH; d = **1** or **2**, thiophenol or *p*-chlorothiophenol, $K_2CO_3$ (1:4:4), EtOH.

2-Chloromethyl-7-nitroimidazo[5,1-*b*]-2,3-dihydrooxazole (**1**) and its 5-methyl derivative (**2**) were synthesized starting from 4,5-dinitroimidazole and 2-methyl-4,5-dinitroimidazole by known procedure [21]. Compounds (**3-16**) were synthesized by the nucleophilic substitution reaction of substrates **1,2** with thiophenol, p-chlorothiophenol, morpholine and piperidine. In the preliminary experiment, reaction has been conducted according to known manner [22], where authors described the nucleophilic substitution of chlorine atom in isomeric 2-chloromethyl-6-nitroimidazo[2,1-*b*]-2,3-dihydrooxazole using equimolar amounts of substrates (nitroimidazodihydrooxazole: thiophenol: $K_2CO_3$ 1:1:1) in DMF. In our experiment, after 10 minutes of stirring in room temperature, analysis of TLC plate indicated that two products were formed. After their separation on silicagel by the column chromatography, compounds: **3** in 25% yield and **9** in 18% yield have been isolated. The reaction was improved by using ethanol as a solvent. After 30 min. of stirring in room temperature, 3-chloro-1-(5-thiophenoxy-4-nitroimidazole-1-yl)propan-2-ol (**3**) was the main product. This compound has been obtained in 57% yield. Comparable yield (59%) of product **3** was observed when the proportion of substrates has been increased to 1:2:2.

Further prolongation of time of these reactions has not improved significantly the quantity of products. Equimolar ratio of nitroimidazooxazole, thiophenol and $K_2CO_3$ favourably led to compound with thiopheno group at C-5 position of nitroimidazole ring, as a result of ring opening and $S_N$ reaction. Only refluxing for 1h of reaction mixture as well as fourfold increase in the amount of thiophenol and basic agent gave the product of nucleophilic substitution at C-5 of imidazole ring and, additionally, chlorine atom in 2-hydroxypropyl chain has been replaced with second thiophenoxy group. As a result 3-thiophenoxy-1-(5-thiophenoxy-4-nitroimidazole-1-yl) propan-2-ol (**9**) has been obtained in good yield (82%). So, we have observed that close structural isomers of nitroimidazodihydrooxazoles react with nucleophiles in different ways giving products with unmodified bicyclic system in case of [2,1-*b*] isomers [22], or compounds with alkyl chain formed after spontaneous dihydrooxazole ring opening reaction which is observed for [5,1-*b*] imidazooxazoles. In continuation of the study, p-chlorothiophenol and secondary cyclic amines (morpholine, piperidine) were also subjected to reactions with nitroimidazodihydro oxazoles (**1**,**2**) in the presence of $K_2CO_3$ (Table 1).

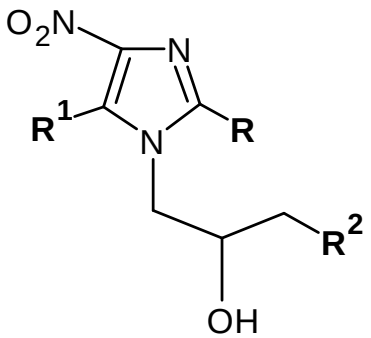
**Table 1:** Reaction conditions used for the synthesis of compounds 3-16

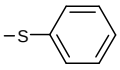
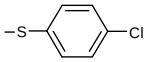
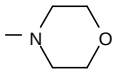
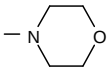
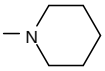
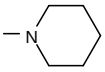
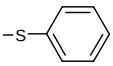
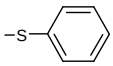
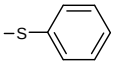
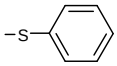
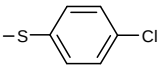
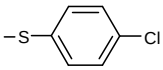
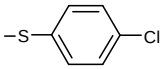
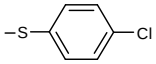
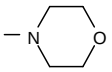
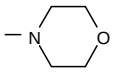
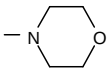
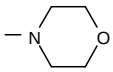
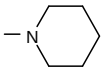
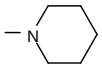
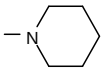
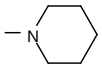
| **Starting materials (proportions)** | **Solvent** | **Products** | **Yield [%]** |
|---|---|---|---|
| (**1**): thiophenol: $K_2CO_3$ (1:1:1) | DMF | **(3)** + **(9)*** | 25 + 18 |
| (**1**): thiophenol: $K_2CO_3$ (1:1:1) | EtOH | **(3)** | 57 |
| (**2**): thiophenol: $K_2CO_3$ (1:1:1) | EtOH | mixture of products | - |
| (**1**): thiophenol: $K_2CO_3$ (1:2:2) | EtOH | **(3)** | 59 |
| (**1**): thiophenol: $K_2CO_3$ (1:4:4) | EtOH | **(9)*** | 82 |
| (**2**): thiophenol: $K_2CO_3$ (1:4:4) | EtOH | **(10)*** | 81 |
| (**1**): *p*-chlorothiophenol: $K_2CO_3$ (1:1:1) | EtOH | **(4)** | 70 |
| (**2**): *p*-chlorothiophenol: $K_2CO_3$ (1:1:1) | EtOH | mixture of products | - |
| (**1**): *p*-chlorothiophenol: $K_2CO_3$ (1:4:4) | EtOH | **(11)*** | 86 |
| (**2**): *p*-chlorothiophenol: $K_2CO_3$ (1:4:4) | EtOH | **(12)*** | 82 |
| (**1**): morpholine: $K_2CO_3$ (1:1:1) | EtOH | **(5)** | 65 |
| (**2**): morpholine: $K_2CO_3$ (1:1:1) | EtOH | **(6)** | 75 |
| (**1**): morpholine: $K_2CO_3$ (1:4:4) | EtOH | **(13)*** | 34 |
| (**2**): morpholine: $K_2CO_3$ (1:4:4) | EtOH | **(14)*** | 40 |
| (**1**): piperidine: $K_2CO_3$ (1:1:1) | EtOH | **(7)** | 65 |
| (**2**): piperidine: $K_2CO_3$ (1:1:1) | EtOH | **(8)** | 72 |
| (**1**): piperidine: $K_2CO$ (1:4:4) | EtOH | **(15)*** | 40 |
| (**2**): piperidine: $K_2CO_3$ (1:4:4) | EtOH | **(16)*** | 61 |

*****- products of disubstitution reaction

Moreover, the influence of methyl group at C-2 position at imidazole ring on the reaction direction has been determined. In most cases, the presence of C-2 methyl group caused increase in the yield of obtained products. Only some attempts of gaining 2-methyl analogs of compounds: 3-chloro-1-(4-nitro-5-thiophenoxyimidazol-1-yl) propan-2-ol and 3-chloro-1-(4-nitro-5-(p-chlorothiophenoxy) imidazol-1-yl)propan-2-ol failed. These reactions led to mixtures of many products with similar $R_f$ values. Results of the other syntheses with use of p-chlorothiophenol and cyclic amines gave products analogous to described compounds (**3**) and (**9**) (Table 2).

**Table 2:** Structure and physical properties of compounds 3-16

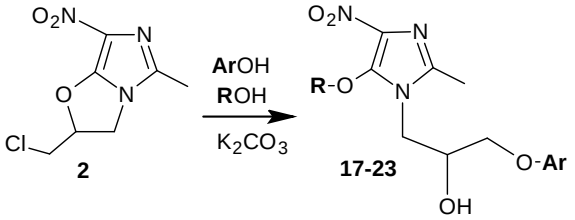


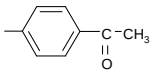
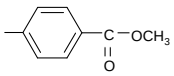
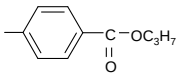
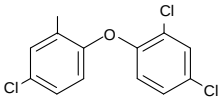
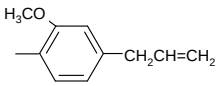
| Compound | R | $R^1$ | $R^2$ | Mp. [°C] | $R_f$* | Yield after cryst [%] |
|---|---|---|---|---|---|---|
| **3** | -H |  | -Cl | 92-93 | 0.65 | 57 |
| **4** | -H |  | -Cl | 124-126 | 0.72 | 70 |
| **5** | -H |  | -Cl | 124-126 | 0.50 | 65 |
| **6** | -$CH_3$ |  | -Cl | 185-186 | 0.53 | 75 |
| **7** | -H |  | -Cl | 127-128 | 0.56 | 65 |
| **8** | -$CH_3$ |  | -Cl | 158-159 | 0.62 | 72 |
| **9** | -H |  |  | 59-61 | 0.83 | 82 |
| **10** | -$CH_3$ |  |  | 51-53 | 0.86 | 81 |
| **11** | -H |  |  | 65-67 | 0.64 | 86 |
| **12** | -$CH_3$ |  |  | 95-96 | 0.72 | 82 |
| **13** | -H |  |  | 110-112 | 0.34 | 34 |
| **14** | -$CH_3$ |  |  | 152-154 | 0.37 | 40 |
| **15** | -H |  |  | 58-60 | 0.48 | 40 |
| **16** | -$CH_3$ |  |  | 129-130 | 0.54 | 61 |

*eluent: $CH_2Cl_2$ : $CH_3OH$ (9:1) on $SiO_2$

Phenols and potassium carbonate were used in 1:2:2 proportion. Depending on the alcohol used in reaction as a solvent, different products were obtained. It was found that alcohol molecule took part in these reactions - alkoxy group is substituted at C-5 in imidazole ring as a result of nucleophilic substitution. When synthesis was performed e.g. in methanol, 5-metoxy- derivative was gained. In case of these reactions between 5-methyl derivative of nitroimidazodihydrooxazole and phenols it was observed that the presence of methyl group connected with C-5 of bicyclic system decreased the yield of forming products in comparison with analogous syntheses using substrate **1** without this methyl group. Respective data are given in Table 3.

**Table 3:** Structure and physical properties of the synthesized compounds (17 – 23)




| Compound | Ar | R | mp. [°C] | $R_f$* | Yield after cryst. [%] |
|---|---|---|---|---|---|
| **17** |  | $C_2H_5$ | 128-130 | 0.70 | 40 |
| **18** |  | $CH_3$ | 158-160 | 0.69 | 12 |
| **19** | | $C_2H_5$ | 74-75 | 0.69 | 18 |
| **20** | | *n*-$C_3H_7$ | 125-126 | 0.67 | 35 |
| **21** |  | *n*-$C_3H_7$ | 72-74 | 0.83 | 57 |
| **22** |  | $CH_3$ | 138-140 | 0.84 | 39 |
| **23** |  | $C_2H_5$ | 85-86 | 0.66 | 35 |

*eluent: $CH_2Cl_2$ : $CH_3OH$ (9:1) on $SiO_2$

Structures of compounds **24** and **25** are given in Figure 1. They are synthesized strictly as it was described by us in [19]. These products are included in this work due to comparing their antifungal properties with compounds having 2-methyl group.

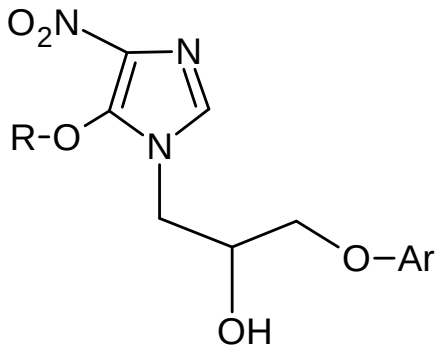



**Figure 1.** Structure of tested compounds **24**, **25**

**24** R = -$CH_3$ Ar = -$C_6H_4COOCH_3$
**25** R = -$C_2H_5$ Ar = -$C_6H_4COCH_3$

**Antifungal activity**

As shown in Table 4, compounds **18, 21, 22** and **16** are highly active against *Trichoderma viride*. Antifungal activity of these compounds was observed even after 21 days of the study. Additionally, moderate anti-Tv activity was observed for products **8** and **17.** Compound **18** showed the high efficiency relative to An, Pc and Pv after 4 days of exposure. The results of visual assessment on 7 and 14 day showed that fungistatic properties decreased significantly. This fact can suggest diffusion of drug into malt agar.

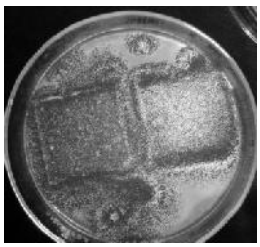
**Table 4:** Fungistatic activity of nitroimidazoles tested against *Aspergillus niger* (An), *Trichoderma viride* (Tv), *Penicillium funiculosum* (Pf), *Paecilomyces variotii* (Pv), expressed as percentage factor of specimen area covered by fungi

| Fungi species | Time of exposition | Compound | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **5** | **7** | **8** | **16** | **17** | **18** | **21** | **22** | **24** | **25** |
| An, Tv, Pc, Pv | 4 days | 4 | 4 | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 4 |
| An | | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 |
| Tv | | 4 | 4 | 1 | 0 | 2 | 0 | 1 | 0 | 2 | 4 |
| Pc | | 4 | 4 | 4 | 4 | 4 | 0 | 4 | 4 | 4 | 4 |
| Pv | | 4 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 4 | 4 |
| An, Tv, Pc, Pv | 7 days | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 |
| An | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Tv | | 4 | 4 | 2 | 1 | 3 | 0 | 1 | 1 | 4 | 4 |
| Pc | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Pv | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| An, Tv, Pc, Pv | 14 days | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| An | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Tv | | 4 | 4 | 3 | 1 | 3 | 1 | 2 | 1 | 4 | 4 |
| Pc | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Pv | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| An, Tv, Pc, Pv | 21days | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| An | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Tv | | 4 | 4 | 4 | 2 | 3 | 1 | 2 | 1 | 4 | 4 |
| Pc | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Pv | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

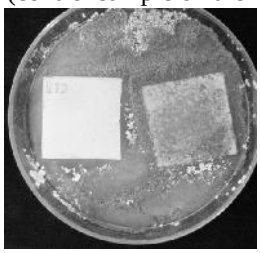
| Index | Rating system |
|---|---|
| 0z | no growth of fungi on the specimen, inhibition zone on the nutrient |
| 0 | no growth of fungi on the specimen |
| 1 | less than 10 % of the specimen area covered by fungi |
| 2 | less than 30 % of the specimen area covered by fungi |
| 3 | less than 60 % of the specimen area covered by fungi |
| 4 | specimen totally overgrown by fungi |

Compound **18** was the most effective nitroimidazole derivative against mixture of fungi. High effectiveness probably was induced by the presence of methyl group at C-2 position of imidazole ring. Moreover, all of 2-$CH_3$ derivatives (**8, 16-18, 21, 22**) are characterized by moderate antifungal activity. Visual results of mycological examination for compound **18** which was exposed against An and Tv are shown in Figure 2.

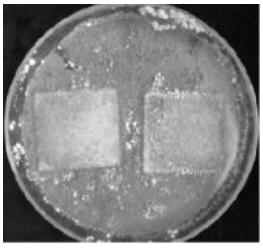
**Figure 2:** Visual results of mycological examination of nitroimidazole derivatives after 7 days (control sample on the right)



Compound **18** infected with An – no antifungal properties – **index 4**



Compound **18** infected with Tv – no growth of fungi – **index 0**



Compound **24** infected with Tv – totally inactive – **index 4**

**Structure – Activity relationships**

Among the 10 derivatives tested, 7 were active as antifungals. The variations at the C-3 position in the N-1 alkyl chain showed that the best results were obtained with phenoxy group having ester substituent (**18, 21**) or with Triclosan rest (**22**). Most active in this series was methyl 4-(2-hydroxy-3-(5-methoxy-2-methyl-4-nitroimidazol-1-

yl)propoxy)benzoate **(18),** very good fungi growth inhibition was observed for compounds **21** and **22.** The introduction of nonpolar, hydrophobic alkenyl chain, instead of ester group, lead to inactive product **23.** Among the secondary, cyclic amine series, good results were gained with the derivatives having two piperidine rings attached to C-3 and C-5 in imidazole system (**16**) and medium results with only one C-3 piperidine rest. Comparing these data with activity of compound with morpholine moiety (**5**), it was observed that only piperidine rest is suitable for this position. Product **5** was totally inactive. In a comparative analysis it was notable that a methyl group at the 2 position of the imidazole ring leads to significant enhancement of antifungal activity. Removal of 2-$CH_3$ reduced biological properties tested. There is no clear evidence about the influence of kind of C-5 substituent. It was found that alkoxy group may be helpful, but it is not essential for good level of antifungal activity. Structure – activity relationships of nitroimidazole derivatives are summarized in Figure 3.

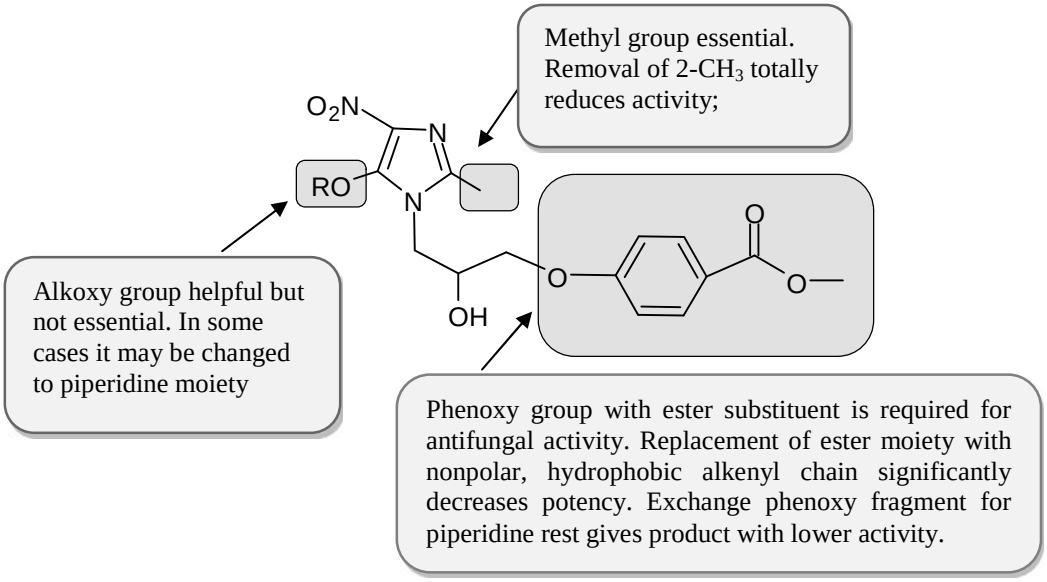



**Figure 3:** SAR study of tested products

***Calculations of clogP and PSA***

Some authors attempted to correlate biological activity with clog P [23]. Lipophilicity and PSA values of synthesized compounds were compared in order to analyze the biological results obtained. Data listed in the Table 5 show that all described products have clog P values ranging from 0.12 to 5.19. Relatively low PSA parameters (87.12 or 96.34 Å) and good enough level of lipophilicity (in most cases clog P < 2) could indicate high pharmacological activity of tested samples [24]. In fact, for compounds **5**, **7**, **24**, **25** such a biological effect has not been observed. Products **8**, **16**, **17**, **21** and **22** show good antifungal properties only against Tv strains in spite of rather favourable both calculated values. Surprisingly, a compound **18** selected as the best antifungal agent from tested series has moderate PSA parameter (128.65 Å) and it has comparable with inactive derivatives clog P value (1.55 Å) so, it could not be found any correlation between both parameters and antifungal activity of tested compounds. It indicates that lipophilicity and PSA of compounds obtained are not only factors responsible for their mode of action.

**Table 5:** The clog P and PSA values for synthesized nitroimidazole derivatives 3-25

| Compound | C log P | PSA [Å$^2$] | Compound | C log P | PSA [Å$^2$] |
|---|---|---|---|---|---|
| **3** | 2.29 | 83.88 | **15** | 1.90 | 90.35 |
| **4** | 2.97 | 83.88 | **16** | 2.02 | 90.35 |
| **5** | 0.63 | 96.34 | **17** | 1.66 | 119.42 |
| **6** | 0.75 | 96.34 | **18** | 1.55 | 128.65 |
| **7** | 1.17 | 87.12 | **19** | 1.93 | 128.65 |
| **8** | 1.82 | 87.11 | **20** | 2.43 | 128.65 |
| **9** | 3.74 | 83.88 | **21** | 3.44 | 128.65 |
| **10** | 3.83 | 83.88 | **22** | 4.99 | 111.56 |
| **11** | 5.10 | 83.88 | **23** | 2.17 | 111.56 |
| **12** | 5.19 | 83.88 | **24** | 1.46 | 128.65 |
| **13** | 0.24 | 108.81 | **25** | 1.57 | 119.42 |
| **14** | 0.12 | 108.81 | | | |

## 4. Conclusion

In the present study, a series of nitroimidazole derivatives have been obtained and their antifungal properties were evaluated. Some of tested products showed significant *in vitro* fungi inhibition against used pathogenic strains. Among examined compounds, methyl 4-(2-hydroxy-3-(5-methoxy-2-methyl-4-nitroimidazol-1-yl)propoxy)benzoate (**18**) has been identified as the best antifungal agent. Other compounds containing a methyl group at the C-2 of the imidazole ring, which showed antifungal activity, must be also taken into consideration. It may be considered promising for the further development of new biologically active substance, especially as the inhibitor of ergosterol synthesis.

## 5. References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