Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 645–652
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis of some *N*-substituted nitroimidazole derivatives as potential antioxidant and antifungal agents

Dorota Olender [a], Justyna Żwawiak [a], Victor Lukianchuk [b], Roman Lesyk [c], Aleksandra Kropacz [d], Andrzej Fojutowski [d], Lucjusz Zaprutko [a,*]

[a] *Department of Organic Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland*
[b] *Department of Pharmacology, Lugansk State Medical University, 50 Years Defence Lugansk 1, 91045 Lugansk, Ukraine*
[c] *Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, 79010 Lviv-10, Ukraine*
[d] *Department of Environmental Protection and Wood Conservation, Wood Technology Institute, Winiarska 1, 60-654 Poznań, Poland*

Received 18 December 2007; received in revised form 17 May 2008; accepted 21 May 2008
Available online 28 May 2008

Abstract

Some new nitroimidazole derivatives have been synthesized by treating 4,5-dinitro- and 2-methyl-4,5-dinitroimidazoles with epoxypropane, epichlorohydrin or phenacyl bromide in alkylation reactions. The nitro group in *N*-substituted 4,5-dinitro- and 2-methyl-4,5-dinitroimidazoles has been replaced with primary and secondary amines to afford 4-amino-5-nitroimidazole derivatives. Some of the compounds have been tested for their antioxidant and antifungal properties against fungi species acting on timber. Nearly all of them have shown significant antioxidant activity in comparison with that of tocopherol, which is used as a reference substance. Two compounds from those tested have revealed very strong fungistatic activity against *Sclerophoma pityophila*.

Keywords: Nitroimidazole; Aminonitroimidazole; Nitro group substitution; Antioxidant activity; Antifungal activity

1. Introduction

For a few decades, nitroimidazoles have been the subject of much interest because of their properties. Depending on the nature of substituents and the position of the nitro group, the nitroimidazole derivatives can show various pharmacological activities [1]. The compounds with nitro group at position 4 are usually less active than the corresponding 5-nitro derivatives. Nitroimidazoles, such as metronidazole, misonidazole, ornidazole, secnidazole, etanidazole and tinidazole, are commonly used as therapeutic agents against a variety of protozoan and bacterial infections of humans and animals [2–4]. It has been suggested by many authors that nitroimidazoles containing nitroimidazooxazole or nitroimidazooxazine structures (CGI-17341, PA-824) might be potential antitubercular agents [5–7]. Derivatives of 5-nitroimidazoles have been tested in cell-based assays and in enzyme assays against HIV-1 recombinant reverse transcriptase [8,9]. 2-Nitroimidazoles play a major role as bioreductive markers for tumour hypoxia and as radiosensitizers [10–12]. Some of them demonstrate antiprotozoan activity [4].

Our earlier investigations have provided evidence of the antifungal and antibacterial properties of *N*-phenacyl-4,5-dinitroimidazole derivatives [13]. Some dinitro- and mononitroimidazole derivatives have been predicted as notable radiosensitizers, antiprotozoal and antibacterial or antiepileptic agents [14].

Antifungal agents are regarded as efficient drugs with relatively low toxicity. New, effective antifungal substances are still being sought because of the increasing number of resistant fungi being isolated.

Antioxidants play a significant role in several important biological processes such as immunity, protection against tissue

* Corresponding author. Tel.: +48 61 8546 670; fax: +48 61 8546 680.
E-mail address: zaprutko@ump.edu.pl (L. Zaprutko).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.05.016

damage, reproduction and growth or development. They preserve adequate function of cells against homeostatic disturbances such as those caused by septic shock, aging and, in general, processes involving oxidative stress. These substances are classified according to their mode of action. Important antioxidants include the chain-breaking or scavenging substances (vitamins E, C and A, bilirubin), preventative (albumin, lactoferrin, haptoblobin) and enzyme antioxidants (catalase and glutathione peroxidase) [15]. They reduce damage to cells and biochemicals caused by free radicals, which are normal products of metabolism. Antioxidants can prevent cardiovascular disease, cancer, cataracts and various other ailments associated with aging [16,17]. The studies suggest that supplementation with antioxidants may be useful in the prevention and treatment of Parkinson's disease [18,19]. Oxidative stress is also important in the pathogenesis of Alzheimer's disease. The studies suggest that supplementation with vitamin E might delay the development of Alzheimer's disease [20,21].

The broad spectrum of biological activity of nitro compounds was the inspiration to test them for antioxidant and antifungal activities. The results obtained in this study can be useful for the design and synthesis of new substances with antioxidant and antifungal activities.

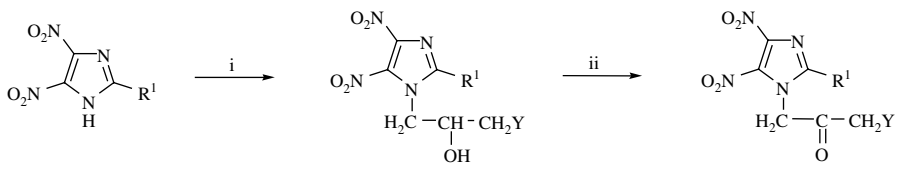
The aim of this study was to determine in vitro the antioxidant properties of some *N*-substituted 4,5-dinitroimidazoles and their 2-methyl derivatives as well as respective compounds with primary or secondary amino group in *C*-4 position of the nitroimidazole ring. In addition some derivatives were tested for their antifungal activity against fungi species attacking timber. Wood can be protected from the attack of fungi decay by applying suitable chemical preservatives. The antioxidative activity experiments were carried out in comparison with tocopheryl acetate.

2. Results and discussion

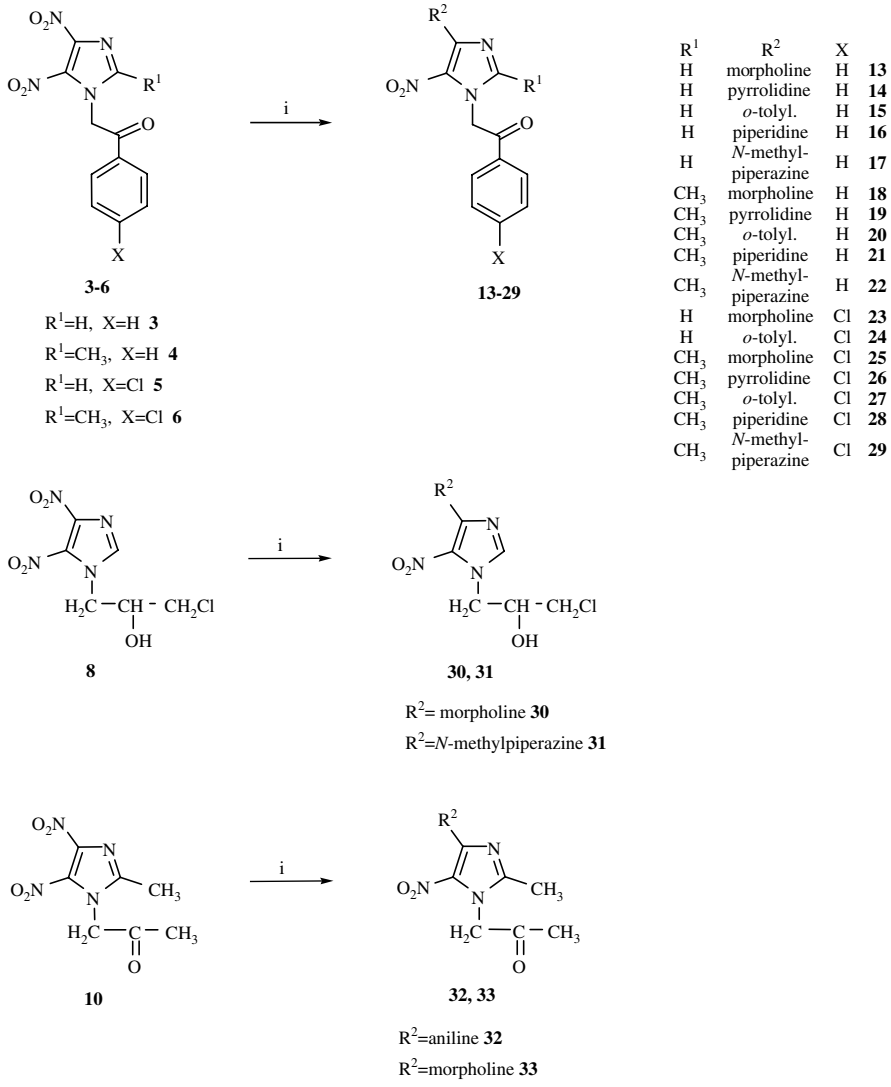
2.1. Chemistry

As starting materials 4,5-dinitroimidazole **1** and 2-methyl-4,5-dinitroimidazole **2** were used [22]. 4,5-Dinitro-1-phenacylimidazole derivatives **3**–**6** of above-mentioned dinitroimidazoles are known substances [13]. *N*-Hydroxypropyl derivatives **7**–**9** were synthesized by the reaction of dinitroimidazoles **1** or **2** with propylene oxide or with epichlorohydrin without solvent or in alcoholic solutions (Scheme 1). The reagents were heated under reflux for about 3 h. The treatment of **2** with an excess of epoxypropane (1:2) led to new *N*-(2-hydroxypropyl) derivative **7**. While reacting **1** with epoxypropane in ethanol or without solvent, formation of respective hydroxypropyl derivative of 4,5-dinitroimidazole was not observed. Dinitroimidazoles **1** and **2** alkylated with epichlorohydrin formed compounds **8** and **9**, respectively [13]. If a methyl group at *C*-2 position of dinitroimidazole ring was present, the solvent was not necessary but formation of **8** in the reaction without solvent was not observed. Thus, the presence of a methyl group at *C*-2 position of the imidazole ring significantly influenced *N*-alkylation and other substitution reactions. Subsequently, derivatives **7**–**9** were transformed into new *N*-(2-oxopropyl)-4,5-dinitroimidazoles **10**–**12** (Scheme 1). The process was carried out using Jones reagent in the oxidation reaction, at room temperature and by using acetone as a solvent. From this reaction mixture the isolated and purified derivatives of ketones were readily obtained. ^{1}H NMR spectra of **10**–**12** showed only methyl and methylenic protons assigned to the side chains or proton at *C*-2 position of the imidazole ring, but no signals of the hydroxyl groups. IR spectra of the compounds prepared showed strong bands in the region 1725–1720 cm^{-1} attributed to the carbonyl group.

Derivatives **3**–**11** were applied to obtain some new products in the reaction with primary and secondary amines such as *o*-toluidine, morpholine, pyrrolidine, piperidine and *N*-methylpiperazine. These reactions were carried out in THF, at room temperature [23]. The treatment of **3**–**6** with the above-mentioned amines, even at molar ratio 1:5 led to the respective 4-amino-5-nitroimidazole derivatives **13**–**29** only (Scheme 2). Formation of aminonitro compounds occurs very easily and often with high yield. The other solvents (ethanol, acetonitrile, methylene chloride) were unsuitable for the reaction. Under these conditions, the amino products were formed in longer time and often with poor yields and purity. With ethanol being used as a solvent, the reaction led to a mixture of both possible isomers: 4-amino-5-nitro- and 5-amino-4-nitroimidazoles. Moreover, elevated temperatures favour the formation of by-products. Derivatives **13**–**17**, **20**, **22**–**24**, **27** and **29** are newly synthesized substances but products **18**, **19**, **21**, **25**, **26** and **28** have been described earlier [23]. IR spectra of some newly synthesized aminonitroderivatives



Scheme 1. Synthesis of *N*-substituted 4,5-dinitroimidazole derivatives. Reagents and conditions: (i) epoxypropane or epichlorohydrin, without solvent or in EtOH, boiling, 2.5–3.5 h; (ii) Jones reagent, acetone, r.t.



R^1	R^2	X	
H	morpholine	H	**13**
H	pyrrolidine	H	**14**
H	*o*-tolyl.	H	**15**
H	piperidine	H	**16**
H	*N*-methyl-piperazine	H	**17**
CH_3	morpholine	H	**18**
CH_3	pyrrolidine	H	**19**
CH_3	*o*-tolyl.	H	**20**
CH_3	piperidine	H	**21**
CH_3	*N*-methyl-piperazine	H	**22**
H	morpholine	Cl	**23**
H	*o*-tolyl.	Cl	**24**
CH_3	morpholine	Cl	**25**
CH_3	pyrrolidine	Cl	**26**
CH_3	*o*-tolyl.	Cl	**27**
CH_3	piperidine	Cl	**28**
CH_3	*N*-methyl-piperazine	Cl	**29**

Scheme 2. Synthesis of *N*-substituted 4-amino-5-nitroimidazole derivatives. Reagents and conditions: (i) amine, THF, r.t., 4–24 h.

(**15**, **20**, **24**, and **27**) show characteristic absorption bands at about 3300–3240 cm^{-1} and near 1600 cm^{-1}. These bands correspond to the N–H groups. Besides, the signal near 1700 cm^{-1} corresponds to a carbonyl group. The absorption bands of nitro groups were observed at ranges 1560–1500 cm^{-1} and 1360–1305 cm^{-1}. The corresponding very strong molecular ions [M^+] were detected in the mass spectra of all synthesized compounds. The fragmentation way exhibited a loss of a nitro group and formation of the ion [$M^+ - 46$] in all aminonitroderivatives. Besides the fragmentation ions, two strong fragments (*m*/*z* 100%) were observed, the first one corresponding to the ion at *m*/*z* 105 originating from the phenacyl group (**13**–**22**) and the second one corresponding to its *p*-chloro substituted derivatives (*m*/*z* 139; **23**–**29**). ^{1}H NMR spectra of new compounds are also in agreement with their molecular formula, for instance the spectra of the derivatives prepared from *o*-toluidine (**15**, **20**, **24** and **27**) show a singlet in the range 9.27–9.56 ppm, assigned to the proton attached to the amine nitrogen atom. The compounds obtained in this work are 4-amino-5-nitroimidazoles. A comparison of the ^{1}H NMR spectra of 5-amino-4-nitroimidazoles reported in literature [24,25] with the spectra of the compounds studied reveals significant differences allowing the empirical differentiation of the isomeric compounds. In the ^{1}H NMR spectra of 4-amino-5-nitro derivatives the signals of the CH_2 protons are shifted towards lower values and are in the range of 5.65–5.80 ppm. This suggests that the amino groups in compounds **13**–**29** are present in the *C*-4 position of the heterocyclic ring. The signals of other H atoms are similar and could not be related with structure, but were observed in the expected regions. Finally, the structure of 4-amino-5-nitroimidazoles was confirmed by X-ray analysis [23]. Substitution mechanism of only one nitro group among two or three, e.g. for *o*-dinitrobenzene derivatives [26] and 2,4,6-trinitrobenzonitrile [27] was described earlier. The reason for C-4 nitro group substitution in *N*-phenacyl-4,5-dinitroimidazoles is non-equivalence of these nitro groups. The considerable reduction of C-5–NO_2 distance is observed with respect to the C-4–NO_2 bond length. This indicates weak conjugation between the C-5 nitro group and the imidazole ring. Moreover,

the C-4 nitro group is significantly deflected and forms a dihedral angle of about 46° with the plane of the imidazole ring [28]. This deflection also favoured C-4 nitro group towards the nucleophilic substitution with amines.

Under the conditions used for preparing *N*-phenacyl derivatives of 4-amino-5-nitroimidazoles, some *N*-(2-hydroxypropyl) and *N*-(2-oxopropyl) derivatives were also subjected to reactions with amines. Similar products were formed on reacting respective 1-(3-chloro-2-hydroxypropyl)-4,5-dinitroimidazole (**8**) with morpholine and *N*-methylpiperazine (**30**,**31**) or 2-methyl-4,5-dinitro-1-(2-oxopropyl)-imidazole (**10**) with aniline and morpholine (**32**,**33**). The molar ratio of dinitroimidazole and amine (1:2.2–1:5) and the type of amines used had essential influence on the structure and quantity of the product obtained. The reaction of dinitroimidazoles with amine in a molar ratio of 1:2.2 led to a mixture of products in the form of oils. It is worth noting that the size and nature of the *N*-1 substituent determined the susceptibility of the imidazole nitro group in the substitution reactions with amines. *N*-(3-Chloro-2-hydroxypropyl) and *N*-(2-oxopropyl) derivatives in the reaction with amines gave mixtures of isomeric substitution products with prevalence of the respective 4-amino-5-nitro-isomers or sometimes diaminoimidazoles [13]. These reactions proceeded more readily and unidirectionally when the molar ratio of dinitroimidazole and amine was 1:5. The reaction gave the desired product in good yield in each case.

The general synthetic pathways are given in Schemes 1 and 2.

Some experimental data for new compounds are tabulated in Table 1.

2.2. Antioxidant activity

All compounds were tested for their in vitro antioxidant activity using the method of non-enzymatic initiation of lipid peroxidation by Fe^{2+} ions and determination of thiobarbituric acid reactive substances (TBARS) assay [29]. The lipid peroxidation (LPO) inhibitory activity of each compound was calculated from the appropriate equation under Section 3.2. and the results were expressed as % of antioxidant activity (AOA) relative to the control blank probe (Table 2). Tocopheryl acetate was used as a reference substance.

As shown in Table 2, compounds **5**, **6**, **8**, **11**, **13**, **14**, **21** and **30** show the strongest antioxidant properties at closing time of the experimental model used. They are more effective than the reference substances. It is also noted that three compounds: **15**, **20** and **24** with *o*-toluidine substituent showed negative or very low values of AOA. These compounds can act as strong oxidant agents under these conditions. The most active were the derivatives bearing chlorine atom in 2-hydroxypropyl and 2-oxopropyl chains or phenacyl group at *N*-1 position of the imidazole ring.

2.3. Antifungal activity

Compounds **5**, **6**, **13**, **21**, **26** and **28** were tested for their antifungal activity using the standard nutrient method against *Sclerophoma pityophila*. This fungus species is one of the most destructive basidiomycetes in coniferous forests and it is the main cause of needle disease in pine thickets. The results of the antifungal activity are given in Table 3 and are expressed as the ED_{50} (substance concentrations retarding the fungal growth rate by 50% in comparison with plates where the agent studied was absent), the effective dose ED_{100} (substance concentrations retarding the fungal growth rate by

Table 1
Yields and basic physical properties of nitroimidazole derivatives

Compound	Yield (%)	m.p. (°C)	R_f	Crystals
7	71	153–155	0.60	Light yellow needles
10	51	90–93	0.60	Light yellow needles
11	74	158–160	0.30	Yellow crystals
13	82	192–194	0.51	Light yellow needles
14	42	166–167	0.60	Yellow needles
15	71	204–207	0.77	Yellow needles
16	67	135–138	0.65	Yellow needles
17	45	126–129	0.26	Yellow needles
20	77	165–168	0.83	Orange needles
22	27	155–157	0.30	Light yellow needles
23	88	192–194	0.53	Yellow needles
24	35	242–244	0.77	Yellow needles
27	80	215–218	0.80	Orange plates
29	82	178–180	0.30	Yellow needles
30	78	150–151	0.75	Yellow needles
31	54	161–163	0.21	Yellow needles
32	65	193–195	0.57	Yellow crystals
33	44	124–126	0.46	Yellow needles

Table 2
Antioxidant activity of nitroimidazole derivatives

Compound	AOA (%)		
Tocopheryl acetate	18.9 ± 1.8	17.8 ± 1.8	18.9 ± 1.8
3	40.6 ± 1.3	39.8 ± 1.5	34.5 ± 1.9
4	26.4 ± 1.4	35.3 ± 1.5	33.8 ± 2.3
5	64.1 ± 0.8	60.0 ± 1.6	67.8 ± 0.9
6	60.2 ± 0.8	57.6 ± 1.8	71.7 ± 1.2
8	56.5 ± 1.6	79.9 ± 1.0	78.6 ± 1.1
10	13.1 ± 1.2	25.8 ± 0.7	25.4 ± 1.2
11	63.7 ± 1.6	76.2 ± 0.7	83.1 ± 0.9
13	56.3 ± 1.6	67.7 ± 1.5	63.8 ± 2.0
14	61.8 ± 2.9	44.2 ± 1.3	80.1 ± 1.1
15	2.9 ± 2.6	7.4 ± 1.5	1.2 ± 2.8
18	45.6 ± 2.9	39.9 ± 1.8	50.9 ± 1.4
19	45.2 ± 0.7	54.1 ± 1.1	61.9 ± 2.0
20	−354.8 ± 11.9	−14.8 ± 2.9	7.3 ± 3.2
21	48.6 ± 2.8	65.9 ± 1.3	66.5 ± 1.5
22	13.1 ± 1.2	17.7 ± 1.9	13.0 ± 2.3
23	37.3 ± 2.3	21.0 ± 2.1	25.3 ± 1.4
24	−1.5 ± 6.6	−31.9 ± 4.0	−35.3 ± 3.7
25	35.9 ± 2.1	43.0 ± 0.8	30.1 ± 2.1
26	20.8 ± 2.5	39.0 ± 2.1	37.1 ± 2.3
27	12.2 ± 1.9	11.8 ± 1.9	13.0 ± 1.3
28	39.9 ± 2.3	36.9 ± 0.8	41.6 ± 1.4
29	37.1 ± 1.8	47.0 ± 0.9	53.2 ± 1.5
30	51.6 ± 1.8	68.8 ± 1.0	69.7 ± 2.7
31	39.1 ± 3.9	62.7 ± 1.3	60.9 ± 1.8
32	17.5 ± 2.7	15.1 ± 1.2	27.4 ± 2.9
33	39.9 ± 4.8	23.2 ± 1.5	23.1 ± 1.3

Table 3
Antifungal activity of nitroimidazole derivatives

Compound	ED_{50}	ED_{100}	LD
5	1000–2500	>5000	>5000
6	500–750	>5000	>5000
13	<25	<25	>5000
21	>5000	>5000	>5000
26	>5000	>5000	>5000
28	<25	<25	>5000

100% in comparison with plates where the agent studied was absent), and LD values (concentrations causing death of inoculum) of the compounds examined.

As shown in Table 3, all compounds are weakly active against the fungus used. On the other hand, derivatives **13** and **28** showed high fungistatic activity (EDs < 25) against *S. pityophila*. High effectiveness was induced by the displacement of nitro group at 4-position on the imidazole ring to morpholine or piperidine and by the presence of a chlorine atom at 4-position on the phenyl ring. The results tabulated in Table 3 show that all compounds caused the death of the fungi studied with a lethal dose (LD) up to 5000 ppm.

3. Experimental

3.1. Chemistry

Melting points were determined on a Boetius apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 300 VT spectrometer (300 MHz). Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, using $CDCl_3$, DMSO-d_6 and CD_3OD as solvents. Coupling constants (J values) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. MS spectra were recorded on a 402 AMD INTECTRA apparatus by the electron impact technique, operating at 75 eV. Infrared (IR) spectra were recorded in KBr tablets using a Specord 75-IR spectrophotometer and were expressed in cm^{-1} scale. The progress of reactions and purity of products were controlled by thin-layer chromatography (TLC) method on silica gel plates (60 F_{254} from Merck) in a $CHCl_3$/MeOH (9:1, v/v) solvent system at room temperature. The spots on the plates were visualized using UV lamp ($\lambda = 254$ nm). Solid products were purified by the crystallization process. Analytical data of C, H, N assays for new compounds were within less than $\pm 0.3\%$ of the theoretical values and are in good agreement with the proposed structures. From among substances, which were used as substrates, the epichlorohydrin, the epoxypropane and the amines were commercial products.

3.1.1. 1-(2-Hydroxypropyl)-2-methyl-4,5-dinitroimidazole (7)

A mixture of **2** (1.72 g, 10 mmol) and epoxypropane (1.40 mL, 1.16 g, 20 mmol) was heated under reflux for about 3 h. The mixture was poured into ice water (60 mL) and stirred. The precipitate was filtered off, air-dried and crystallized from 40% ethanol, yielding 1.63 g of **7** (71%).

IR: 3305 (ν OH); 1525, 1340 (ν C–NO_2); 1115 (ν C–OH). MS: *m/z* (rel. int.%) = 230 (3.5) M^+, 215 (2.3), 183 (100.0), 166 (3.1), 156 (40.6), 111 (39.3), 82 (33.6). ^{1}H NMR (DMSO-d_6): $\delta = 5.25$ (s, 1H, OH), 4.30 and 4.05 (two m, 2H, CH_2), 3.90 (m, 1H, CH), 2.49 (s, 3H, CH_3–Im), 1.13 (d, $J = 6.0$, 3H, CH_3-chain).

3.1.2. General procedure for the preparation of 1-(2-oxopropyl)-derivatives of 4,5-dinitroimidazole

To a solution of respective *N*-hydroxypropyl derivative **7**, **8** or **9** (10 mmol) in acetone (100 mL), oxidising Jones reagent (10 mL) was dropped at room temperature. After 24 h isopropanol (10 mL) was added. The dark green precipitate was filtered and washed with a small volume of acetone. The combined filtrates were poured into water (250 mL) and the solution was held for 2–3 days to afford the crystalline solid. The crude product was filtered, washed with water, air-dried and crystallized from methanol to yield **10**, **11** or **12**.

*3.1.2.1. 2-Methyl-4,5-dinitro-1-(2-oxopropyl)-imidazole (**10**).* IR: 1725 (ν C=O); 1525, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 228 (1.1) M^+, 182 (2.0), 156 (9.2), 93 (2.8), 43 (100.0). ^{1}H NMR (DMSO-d_6): $\delta = 5.39$ (s, 2H, N–CH_2), 2.41 (s, 3H, CH_3–Im), 2.30 (s, 3H, $COCH_3$).

*3.1.2.2. 1-(3-Chloro-2-oxopropyl)-4,5-dinitroimidazole (**11**).* IR: 1720 (ν C=O); 1500, 1305 (ν C–NO_2). MS: *m/z* (rel. int.%) = 250 (1.8, $M^+ + 2$), 248 (4.1) M^+, 202 (5.9), 172 (20.6), 142 (43.1), 77 (100.0). ^{1}H NMR (DMSO-d_6): $\delta = 8.10$ (s, 1H, 2-Im), 5.56 (s, 2H, N–CH_2), 4.78 (s, 2H, CH_2Cl).

*3.1.2.3. 1-(3-Chloro-2-oxopropyl)-2-methyl-4,5-dinitroimidazole (**12**).* IR: 1720 (ν C=O); 1500, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 264 (15.2, $M^+ + 2$), 262 (40.9) M^+, 227 (8.3), 217 (27.5), 186 (18.3), 156 (89.5), 93 (50.3), 77 (100.0). ^{1}H NMR (DMSO-d_6): $\delta = 5.49$ (s, 2H, N–CH_2), 4.78 (s, 2H, CH_2Cl), 2.43 (s, 3H, CH_3–Im).

3.1.3. General procedure for the preparation of aminonitroderivatives (**13–33**)

To a solution of appropriate 4,5-dinitroimidazole or 2-methyl-4,5-dinitroimidazole derivatives **3–6**, **8** or **10** (1 mmol) in THF (5 mL), a respective amine: secondary (morpholine, pyrrolidine, piperidine, or *N*-methylpiperazine) or primary (aniline, *o*-toluidine) (5 mmol) was added slowly and dropwise at room temperature. After left to rest for 4–24 h, the precipitate was filtered off, washed with a small quantity of cold THF and air-dried. The crude solid was crystallized from a mixture of methanol:water (9:1) to yield **13–33**.

*3.1.3.1. 4-(Morpholin-4-yl)-5-nitro-1-phenacylimidazole (**13**).* IR: 1680 (ν C=O); 1540, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 316 (30.2) M^+, 299 (34.1), 271 (62.0), 253 (44.3), 168 (7.1), 105 (100.0), 77 (52.5). ^{1}H NMR ($CDCl_3$): $\delta = 7.98$ (m, 2H, 2,6-Ph), 7.67 (m, 1H, 4-Ph), 7.53 (m, 2H,

3,5-Ph), 7.37 (s, 1H, 2-Im), 5.70 (s, 2H, N–CH_2), 3.85 (m, 4H, 2 × CH_2, 3,5-morpholine), 3.62 (m, 4H, 2 × CH_2, 2,6-morpholine).

*3.1.3.2. 5-Nitro-1-phenacyl-4-(pyrrolidin-1-yl)-imidazole (**14**).* IR: 1680 (ν C=O); 1550, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 300 (24.8) M^+, 283 (68.5), 255 (19.0), 224 (12.4), 197 (10.9), 146 (12.9), 105 (100.0). ^{1}H NMR ($CDCl_3$): δ = 7.98 (m, 2H, 2,6-Ph), 7.64 (m, 1H, 4-Ph), 7.51 (m, 2H, 3,5-Ph), 7.32 (s, 1H, 2-Im), 5.67 (s, 2H, N–CH_2), 3.68 (t, *J* = 6.6, 4H, 2 × CH_2, 2,5-pyrrolidine), 1.98 (m, 2 × CH_2, 3,4-pyrrolidine).

*3.1.3.3. 5-Nitro-1-phenacyl-4-(o-tolylamino)-imidazole (**15**).* IR: 3275 (ν N–H); 1685 (ν C=O); 1600 (σ N–H); 1520, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 336 (63.7) M^+, 305 (8.9), 290 (17.1), 283 (68.5), 170 (8.9), 157 (12.3), 105 (100.0). ^{1}H NMR ($CDCl_3$): δ = 9.28 (s, 1H, NH), 8.27 (d, *J* = 7.9, 1H, 6-tolyl), 8.01 (m, 2H, 2,6-Ph), 7.68 (m, 1H, 4-Ph), 7.55 (m, 2H, 3,5-Ph), 7.47 (s, 1H, 2-Im), 7.28 (m, 2H, 4,5-tolyl), 7.09 (m, 1H, 3-tolyl), 5.77 (s, 2H, N–CH_2), 2.40 (s, 3H, CH_3–tolyl).

*3.1.3.4. 5-Nitro-1-phenacyl-4-(piperidin-1-yl)-imidazole (**16**).* IR: 1680 (ν C=O); 1550, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 314 (20.6) M^+, 297 (53.7), 269 (24.1), 238 (10.1), 183 (9.1), 161 (40.0), 105 (100.0). ^{1}H NMR ($CDCl_3$): δ = 8.01 (m, 2H, 2,6-Ph), 7.64 (m, 1H, 4-Ph), 7.53 (m, 2H, 3,5-Ph), 7.35 (s, 1H, 2-Im), 5.69 (s, 2H, N–CH_2), 3.56 (m, 4H, 2 × CH_2, 2,6-piperidine), 1.73 (m, 6H, 3 × CH_2, 3,4,5-piperidine).

*3.1.3.5. 4-(4-Methylpiperazin-1-yl)-5-nitro-1-phenacylimidazole (**17**).* IR: 1690 (ν C=O); 1520, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 329 (15.9) M^+, 312 (36.6), 284 (22.8), 269 (7.4), 180 (11.7), 162 (29.5), 105 (100.0). ^{1}H NMR ($CDCl_3$): δ = 7.98 (m, 2H, 2,6-Ph), 7.66 (m, 1H, 4-Ph), 7.51 (m, 2H, 3,5-Ph), 7.37 (s, 1H, 2-Im), 5.70 (s, 2H, N–CH_2), 3.67 (t, *J* = 4.9, 4H, 2 × CH_2, 2,6-piperazine), 2.63 (t, *J* = 4.9, 4H, 2 × CH_2, 3,5-piperazine), 2.38 (s, 3H, >N–CH_3).

*3.1.3.6. 2-Methyl-5-nitro-1-phenacyl-4-(o-tolylamino)-imidazole (**20**).* IR: 3300 (ν N–H); 1700 (ν C=O); 1620 (σ N–H); 1540, 1360 (ν C–NO_2). MS: *m/z* (rel. int.%) = 350 (100.0) M^+, 333 (4.4), 319 (11.4), 304 (35.3), 263 (13.1), 157 (22.0), 105 (78.4). ^{1}H NMR ($CDCl_3$): δ = 9.56 (s, 1H, NH), 8.37 (d, *J* = 7.4, 1H, 6-tolyl), 8.02 (m, 2H, 2,6-Ph), 7.66 (m, 1H, 4-Ph), 7.54 (m, 2H, 3,5-Ph), 7.27 (m, 2H, 4,5-tolyl), 7.05 (m, 1H, 3-tolyl), 5.80 (s, 2H, N–CH_2), 2.40 (s, 3H, CH_3–tolyl), 2.39 (s, 3H, CH_3–Im).

*3.1.3.7. 2-Methyl-4-(4-methylpiperazin-1-yl)-5-nitro-1-phenacylimidazole (**22**).* IR: 1700 (ν C=O); 1520, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 343 (12.8) M^+, 326 (52.5), 298 (26.8), 273 (12.6), 228 (10.4), 160 (28.4), 105 (100.0). ^{1}H NMR ($CDCl_3$): δ = 8.01 (m, 2H, 2,6-Ph), 7.66 (m, 1H, 4-Ph), 7.53 (m, 2H, 3,5-Ph), 5.73 (s, 2H, N–CH_2), 3.67 (t, *J* = 4.9, 4H, 2 × CH_2, 2,6-piperazine), 2.58 (t, *J* = 4.9, 4H, 2 × CH_2, 3,5-piperazine), 2.35 (s, 3H, >N–CH_3), 2.32 (s, 3H, CH_3–Im).

*3.1.3.8. 1-(4-Chlorophenacyl)-4-(morpholin-4-yl)-5-nitroimidazole (**23**).* IR: 1670 (ν C=O); 1520, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 352 (12.1, $M^+ + 2$), 350 (28.5) M^+, 333 (25.6), 305 (51.1), 287 (34.1), 168 (11.2), 139 (100.0). ^{1}H NMR ($CDCl_3$): δ = 7.95 (m, 2H, 2,6-Ph), 7.52 (m, 2H, 3,5-Ph), 7.36 (s, 1H, 2-Im), 5.65 (s, 2H, N–CH_2), 3.86 (m, 4H, 2 × CH_2, 3,5-morpholine), 3.63 (m, 4H, 2 × CH_2, 2,6-morpholine).

*3.1.3.9. 1-(4-Chlorophenacyl)-5-nitro-4-(o-tolylamino)-imidazole (**24**).* IR: 3240 (ν N–H); 1670 (ν C=O); 1600 (σ N–H); 1500, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 372 (38.4, $M^+ + 2$), 370 (91.4) M^+, 324 (28.5), 296 (5.5), 185 (15.2), 158 (19.0), 139 (100.0). ^{1}H NMR ($CDCl_3$): δ = 9.27 (s, 1H, NH), 8.27 (d, *J* = 7.7, 1H, 6-tolyl), 7.96 (m, 2H, 2,6-Ph), 7.53 (m, 2H, 3,5-Ph), 7.47 (s, 1H, 2-Im), 7.28 (m, 2H, 4,5-tolyl), 7.07 (m, 1H, 3-tolyl), 5.72 (s, 2H, N–CH_2), 2.40 (s, 3H, CH_3–tolyl).

*3.1.3.10. 1-(4-Chlorophenacyl)-2-methyl-5-nitro-4-(o-tolylamino)-imidazole (**27**).* IR: 3280 (ν N–H); 1690 (ν C=O); 1620 (σ N–H); 1520, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 386 (38.0, $M^+ + 2$), 384 (83.2) M^+, 338 (34.4), 297 (13.6), 269 (8.2), 199 (13.2), 158 (20.2), 139 (100.0). ^{1}H NMR ($CDCl_3$): δ = 9.55 (s, 1H, NH), 8.36 (d, *J* = 7.4, 1H, 6-tolyl), 7.97 (m, 2H, 2,6-Ph), 7.53 (m, 2H, 3,5-Ph), 7.27 (m, 2H, 4,5-tolyl), 7.06 (m, 1H, 3-tolyl), 5.75 (s, 2H, N–CH_2), 2.41 (s, 3H, CH_3–tolyl), 2.39 (s, 3H, CH_3–Im).

*3.1.3.11. 1-(4-Chlorophenacyl)-2-methyl-4-(4-methylpiperazin-1-yl)-5-nitroimidazole (**29**).* IR: 1680 (ν C=O); 1520, 1320 (ν C–NO_2). MS: *m/z* (rel. int.%) = 379 (7.9, $M^+ + 2$), 377 (18.6) M^+, 360 (78.4), 332 (47.8), 307 (15.8), 289 (15.3), 194 (40.2), 139 (100.0). ^{1}H NMR ($CDCl_3$): δ = 7.94 (m, 2H, 2,6-Ph), 7.49 (m, 2H, 3,5-Ph), 5.65 (s, 2H, N–CH_2), 3.66 (t, *J* = 4.9, 4H, 2 × CH_2, 2,6-piperazine), 2.57 (t, *J* = 4.9, 4H, 2 × CH_2, 3,5-piperazine), 2.35 (s, 3H, >N–CH_3), 2.32 (s, 3H, CH_3–Im).

*3.1.3.12. 1-(3-Chloro-2-hydroxypropyl)-4-(morpholin-4-yl)-5-nitroimidazole (**30**).* IR: 3400–3500 (ν O–H); 1520, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 292 (36.3, $M^+ + 2$), 290 (87.4) M^+, 273 (80.9), 245 (82.7), 227 (100.0), 208 (24.4), 154 (24.9), 113 (49.2). ^{1}H NMR ($CDCl_3$): δ = 7.83 (s, 1H, 2-Im), 5.63 (m, 1H, OH), 4.58 (m, 1H, CH), 4.03 (m, 2H, CH_2Cl), 3.54 (m, 10H, 4 × CH_2 morpholine and N–CH_2).

*3.1.3.13. 1-(3-Chloro-2-hydroxypropyl)-4-(4-methylpiperazin-1-yl)-5-nitroimidazole (**31**).* IR: 3400–3500 (ν O–H); 1520, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 305 (10.1, $M^+ + 2$), 303 (24.1) M^+, 286 (49.8), 258 (34.4), 221 (28.7), 180 (11.5), 163 (21.2), 137 (27.7), 70 (100.0). ^{1}H NMR ($CDCl_3$): δ = 7.79 (s, 1H, 2-Im), 5.65 (m, 1H, OH), 4.58 (m,

1H, CH), 4.02 (m, 2H, CH_2Cl), 3.62 (m, 2H, N–CH_2), 3.47 (t, $J = 5.6$, 4H, $2 \times CH_2$, 2,6-piperazine), 2.42 (t, $J = 4.8$, 4H, $2 \times CH_2$, 3,5-piperazine), 2.21 (s, 3H, N–CH_3).

*3.1.3.14. 2-Methyl-5-nitro-4-1-(2-oxopropyl)-phenylaminoimidazole (**32**).* IR: 3275 (ν N–H); 1720 (ν C=O); 1615 (σ N–H); 1520, 1325 (ν C–NO_2). MS: *m/z* (rel. int.%) = 274 (100.0) M^+, 242 (2.0), 228 (7.2), 187 (34.1), 145 (43.2), 104 (50.9), 77 (73.9). ^{1}H NMR ($CDCl_3$): $\delta = 9.43$ (s, 1H, NH), 7.71 (m, 2H, 2,6-Ph), 7.37 (m, 3H, 3,4,5-Ph), 5.12 (s, 2H, N–CH_2), 2.36 (s, 3H, CH_3–Im), 2.30 (s, 3H, $COCH_3$).

*3.1.3.15. 2-Methyl-4-(morpholin-4-yl)-5-nitro-1-(2-oxopropyl)-imidazole (**33**).* IR: 1720 (ν C=O); 1560, 1340 (ν C–NO_2). MS: *m/z* (rel. int.%) = 268 (84.2) M^+, 251 (89.8), 223 (100.0), 205 (86.9), 193 (24.0), 182 (18.1), 152 (19.5), 123 (25.1), 98 (91.1). ^{1}H NMR (CD_3OD): $\delta = 5.20$ (s, 2H, N–CH_2), 3.81 (m, 4H, $2 \times CH_2$, 3,5-morpholine), 3.54 (m, 4H, $2 \times CH_2$, 2,6-morpholine), 2.30 (s, 3H, CH_3–Im), 2.27 (s, 3H, $COCH_3$).

3.2. In vitro antioxidant assay

Antioxidant activity (AOA) of nitroimidazole derivatives was studied by in vitro assays. AOA in modelling system was determined under the conditions of non-enzymatic initiation of lipid peroxidation by Fe^{2+} ions [29]. The suspension of egg lipoproteins (SEL) was used as a substrate and fat-soluble antioxidant – tocopheryl acetate was used as a reference substance.

Free-radical reaction was induced by Fe^{2+} ions and the intensity of the lipid peroxidation processes in model system was estimated by the spectrophotometric determination of thiobarbituric acid (TBA) and their reactants (TBARS) concentration, by taking into account the coefficient of molar extinction for malonodialdehyde (MDA), which is the main lipooxidation product. SEL was prepared by homogenisation of an egg yolk with full volume of the phosphate buffer pH 7.4. The substances investigated (as 10^{-3} mol/mL solution) and 1 mL of 0.7% solution of $FeSO_4 \cdot 7H_2O$ were added to 1 mL of the suspensions. Mixtures were incubated at 37 °C. For each substance three samples were subjected to measurements: the experimental one (containing the investigated compound and iron(II) sulphate), the control (containing iron(II) sulphate only) and the blind test (without Fe^{2+} ions and investigated compounds). Antioxidant activity of nitroimidazole derivatives was investigated after 15, 30 and 60 min since the moment of initiation of the free-radical oxidation. After the intervals mentioned, the reactions were terminated by the addition of 1 mL of 25% trichloroacetic acid solution (containing 2.5 mg Trilone B in 100 mL of solution for linkage of Fe^{2+}). Subsequently, the samples were centrifuged and the supernatants were incubated in an acidic medium with the same volume of thiobarbituric acid solution at 95 °C for 1 h. After cooling, the reaction product was extracted by *n*-butanol–pyridine mixture (15:1 by volume) and the optical density of the organic phase was measured at 532 nm. The antioxidant activities (AOA) (in %) were determined using the formula:

$$\mathrm{AOA} = \frac{C_K - C_C}{C_K} \times 100$$

where:

C_K – concentration of thiobarbituric acid reactants (TBARS) in control assay, nmol/mL;
C_C – concentration of thiobarbituric acid reactants (TBARS) in assay with investigated compound, nmol/mL.

Each determination was repeated three times; the results obtained were subjected to statistical analysis by the student's-*t* test.

3.3. Antifungal assay

In the study performed, the fungal species *S. pityophila*, which is highly aggressive to timber, was used. The strain *S. pityophila* (Corda) v. Höhn, S 231 originated from the collection of Wood Technology Institute, Poznań, Poland.

The fungicidal effectiveness of some of the compounds obtained was determined using the method proposed by Ważny and Thornton [30] and later used in other tests with biocides designed for wood protection [31,32]. The fungal growth rates were measured in 90 mm diameter Petri dishes using the agar dilution test. Nine concentrations of the compounds were studied in a geometric progression from 25 to 5000 ppm. Stock mixtures of each concentration were produced in sterile malt agar (1.5% agar and 4% malt-extract), 20 mL of which was added to each Petri dish. Three replicate plates of each concentration of each chemicals were centrally inoculated with a 5-mm diameter disc taken from the submargin of 10-day-old cultures of the desired test fungus grown on malt agar. The plates were incubated at 22 ± 1 °C in darkness. The duration of the test was determined by waiting for complete plate coverage by the fungus mycelium that is until 12 days for the researched species of *S. pityophila*. If the growth did not begin on the chemical-containing agar after 12 days, the inoculum was removed and transferred to a fresh malt agar plate for determination of the fungal viability. The results obtained were used to calculate ED_{50}, the effective dose ED_{100}, and LD for all compounds examined.

Acknowledgements

This study was partially supported by Polish State Committee for Scientific Research (grant no. 2 PO5F 03927).

References

[1] J.H. Boyer, Nitroazoles, VCH Publishers, Inc., Deerfield Beach, FL, 1986, pp. 165–166 and references cited therein.
[2] N. Ravether, Parasitol. Res. 90 (2003) 19–39.
[3] D.I. Edwards, J. Antimicrob. Chemother. 31 (1993) 9–20.

[4] P.B. Petray, M.J. Morilla, R.S. Corral, E.L. Romero, Mem. Inst. Oswaldo Cruz 99 (2004) 233–235.
[5] C.E. Barry, H.I.M. Boshoff, C.S. Dowd, Curr. Pharm. Des. 10 (2004) 3239–3262.
[6] C.K. Stover, P. Warrener, D.R. Van Devanter, D.R. Sherman, T.M. Arain, M.H. Langhorne, S.W. Anderson, J.A. Towell, Y. Yuan, D.N. McMurray, B.N. Krelswirth, C.E. Barryl, W.R. Baker, Nature 405 (2000) 962–966.
[7] D.R. Ashtekar, R. Costa-Perira, K. Nagarajan, N. Vishvanathan, A.D. Bhatt, W. Rittel, Antimicrob. Agents Chemother. 37 (1993) 183–186.
[8] R. Silvestri, M. Artico, G. De Martino, R. Ragno, S. Massa, R. Loddo, C. Murgioni, A.G. Loi, P. La Colla, A. Pani, J. Med. Chem. 45 (2002) 1567–1576.
[9] R. Silvestri, M. Artico, S. Massa, T. Marceddu, F. De Montis, P. La Colla, Bioorg. Med. Chem. Lett. 10 (2000) 253–256.
[10] R.J. Hodgkiss, Anticancer Drug Des. 13 (1998) 687–702.
[11] H. Hori, C.Z. Jin, M. Kiyono, S. Kasai, M. Shimamura, S. Inayama, Bioorg. Med. Chem. 5 (1997) 591–599.
[12] S. Kasai, H. Nagasawa, M. Yamashita, M. Masui, H. Kuwasaka, T. Oshodani, Y. Uto, T. Inomata, S. Oka, S. Inayamata, H. Hori, Bioorg. Med. Chem. 9 (2001) 453–464.
[13] L. Zaprutko, M. Gajdziński, W. Michalska, K. Pietkiewicz, K. Lutomski, Z. Łukaszewski, U. Wrzeciono, Pharmazie 44 (1989) 817–820.
[14] D. Olender, R. Lesyk, L. Zaprutko, Pol. J. Pharmacol. 56 (Suppl.) (2004) 175.
[15] V.M. Victor, K.J. McCreath, M. Rocha, Recent Patents Anti-Infect. Drug Disc. 1 (2006) 17–31.
[16] K. Sudha, A. Rao, S. Rao, A. Rao, Neurol. India 51 (2003) 60–62; Acc. Chem. Abstr. 141 (2004) 52141w.
[17] B. Halliwell, Drugs Aging 18 (2001) 685–716; Acc. Chem. Abstr. 137 (2002) 15048t.
[18] K. Asplund, J. Intern. Med. 251 (2002) 372–392.
[19] K.N. Prasad, W.C. Cole, B. Kumar, J. Am. Coll. Nutr. 18 (5) (1999) 413–423.
[20] A. Kontush, S. Schekatolina, Ann. N. Y. Acad. Sci. 1031 (1) (2004) 249–262.
[21] C.J. Foy, A.P. Passmore, M.D. Vahidassr, I.S. Young, J.T. Lawson, Q. J. Med. 92 (1999) 39–45.
[22] S.S. Novikov, L.I. Khmel'nitskii, O.V. Lebedev, V.V. Sevast'yanova, L.V. Epishina, Khim. Geterotsikl. Soedin 6 (1970) 503–507.
[23] L. Zaprutko, D. Olender, A. Gzella, Monatsh. Chem. 134 (2003) 1145–1150.
[24] S. Sobiak, Synth. Commun. 28 (1998) 2703–2711.
[25] S. Sobiak, Pol. J. Chem. 72 (1998) 78–83.
[26] M.D. Senskey, J.D. Bradshaw, C.A. Tessier, W.J. Youngs, Tetrahedron Lett. 36 (1995) 6217–6220.
[27] S.A. Shevelev, I.L. Dalinger, T.I. Cherkasova, Tetrahedron Lett. 42 (2001) 8539–8541.
[28] A. Gzella, M.S. Kaczmarek, Z. Wozniak, Acta Crystallogr. E62 (2006) o2168–o2170.
[29] U.I. Gubsky, V.V. Dunaev, I.F. Belenichev, Methods of Evaluation of Antioxidant Activity of Compounds at Free Radical Processes Initiation in Experiments In vitro. Methodical Recommendation, State Pharmacological Center of Ministry of Health of Ukraine, Kyiv, 2002.
[30] J. Ważny, I.D. Thornton, Holzforschung 40 (1986) 383–388.
[31] J. Pernak, J. Zabielska-Matejuk, A. Kropacz, J. Foksowicz-Flaczyk, Holzforschung 58 (2004) 286–291.
[32] J. Pernak, I. Goc, A. Fojutowski, Holzforschung 59 (2005) 473–475.