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Highly Lipophilic Benzoxazoles with Potential Antibacterial Activity

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Abstract: A series of lipophilic 2-substituted 5,7-di-*tert*-butylbenzoxazoles was prepared in average yields by the reaction of 3,5-di-*tert*-butyl-1,2-benzoquinone with amino acids and dipeptides bearing N-terminal glycine. Dipeptides having other N-terminal amino acids undergo oxidative deamination. 5,7-Di-*tert*-butylbenzoxazoles have shown activity against *Mycobacterium tuberculosis* and some nontuberculous strains where isoniazid has been inactive. Antifungal activity was mediocre.

Keywords: 3,5-di-*tert*-Butyl-1,2-benzoquinone, 5,7-di-*tert*-butylbenzoxazoles, antimycobacterial activity, antifungal activity

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Introduction

The objectives of this study were the preparation and biological testing of some highly lipophilic 2-substituted-5,7-di-*tert*-butyl-benzoxazoles. Such lipophilicity may permit their easier penetration through the lipophilic mycobacterial cell walls. Benzoxazoles have been extensively studied for their antibacterial and antifungal activity [1,2], anticancer activity [3], and also as new non-nucleoside

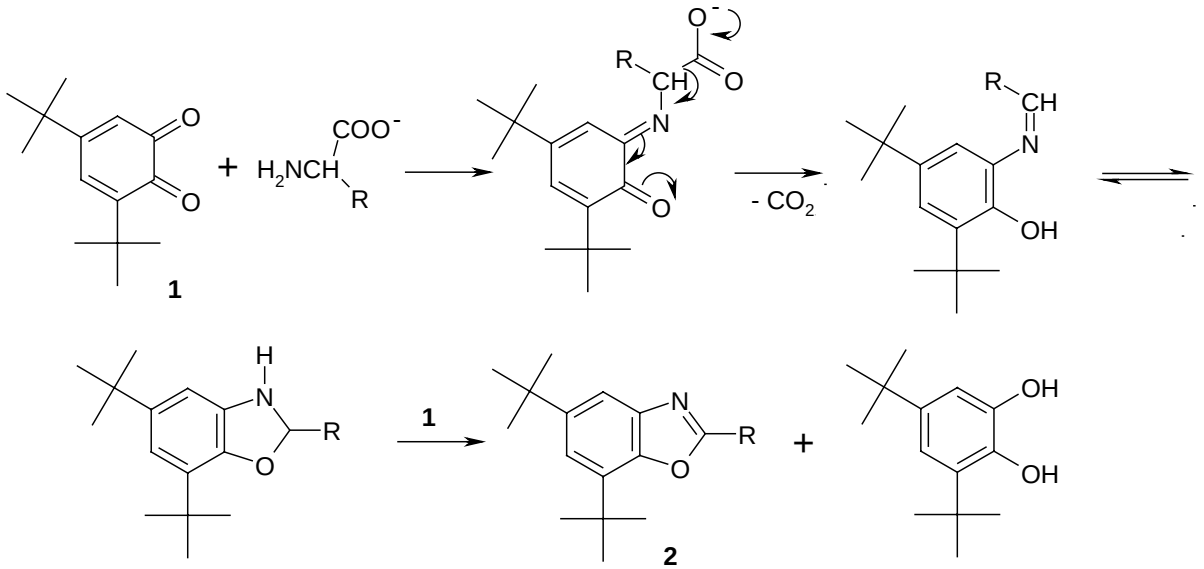
topoisomerase I poisons [4] and HIV-1 reverse transcriptase inhibitors [5,6]. Benzoxazoles are also interesting fluorescent probes which show high Stokes shift and present thermal and photophysical stability due to an excited state intramolecular proton transfer mechanism [7]. They interfere with the biosynthesis of coloured carotenoids by inhibiting the enzyme phytoene desaturase so they are studied as potential bleaching herbicides [8]. Benzoxazoles can be considered as structural isosteres of the naturally occurring nucleic bases adenine and guanine, which allow them to interact easily with polymers of living systems. They have shown low toxicity in warm-blooded animals [9].

For preparation of 2-substituted-5,7-di-*tert*-butylbenzoxazoles the method of choice involves reactions of 3,5-di-*tert*-butyl-1,2-benzoquinone (DTBBQ) with primary alkyl primary amines, amino acids and some of their derivatives [10]. From di- and oligopeptides those with glycine at the N-terminal also form the desired products.

Results and Discussion

Amino acids (Gly, Ala, Phe, Pgl, Val, Leu, Met, Tyr, Trp) and some of their derivatives such as glycinamide and tryptamine produced with DTBBQ the benzoxazole derivatives **2a-j** in average yields. Lysine reacted with both amino groups to form the *bis*(benzoxazole) derivative **2k**. The reaction occurred under mild conditions in ethanol (60-96 %) at a temperature of 50 °C. TLC on silica gel showed a complex mixture of products that were separated by preparative TLC on silica gel plates or by flash column chromatography. All products were characterized by NMR, UV-Vis, IR spectra, elemental analyses and calculated LogP. UV-Vis absorption bands (typical values for **2b**: 208, 236, 274 nm) are useful for benzoxazole identification.

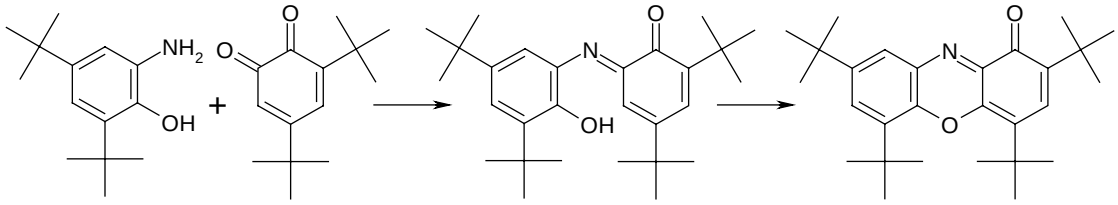
Scheme 1



2a	R = H	**2g**	R = $CH_2CH_2SCH_3$
2b	R = CH_3	**2h**	R = $CH_2C_6H_4$(4-OH)
2c	R = C_6H_5	**2i**	R = *1H*-indol-3-yl(methyl)
2d	R = $CH_2C_6H_5$	**2j**	R = $CONH_2$
2e	R = $CH(CH_3)_2$	**2k**	R = 3-(5,7-di-*tert*-butylbenzoxazol-2-yl)-propyl
2f	R = $CH_2CH(CH_3)_2$		

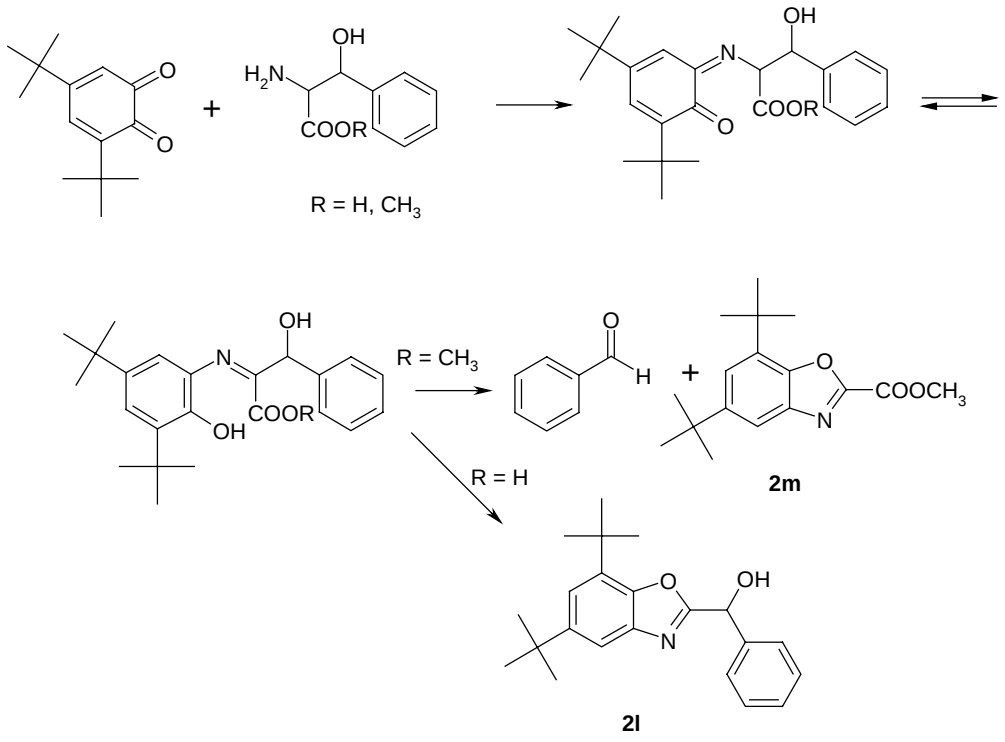
The formation of the target compounds from amino acids and DTBBQ is a multistep process that involves a sequence of consecutive reactions in which the intermediates cannot be detected to confirm the proposed reaction scheme (Scheme 1). In the first step the amino group is added to the less hindered carbonyl group in the position 1 of DTBBQ, followed by dehydration and formation of both *E/Z* isomeric quinone imines. The unstable quinone imines rearrange spontaneously into a mixture of two *E/Z* isomeric Schiff bases that cyclize into a mixture of two 2,3-dihydrobenzoxazole stereoisomers. The latter is dehydrogenated by a second molecule of DTTBQ into a benzoxazole with loss of carbon dioxide (for a review see [10]). The needed amount of the DTBBQ was produced by air oxidation from the 3,5-di-*tert*-butylbenzene-1,2-diol produced from DTBBQ during the dehydrogenation process. The main reaction sequence is accompanied by the formation of highly coloured by-products, especially 2,4,6,8-tetra-*tert*-butylphenoxazine-1-one [11], which complicate isolation of pure benzoxazoles. Formation of these pigments originates in the reaction of the Schiff base or its hydrolysis product, 2-amino-3,5-di-*tert*-butylphenol, with DTBBQ (Scheme 2).

Scheme 2



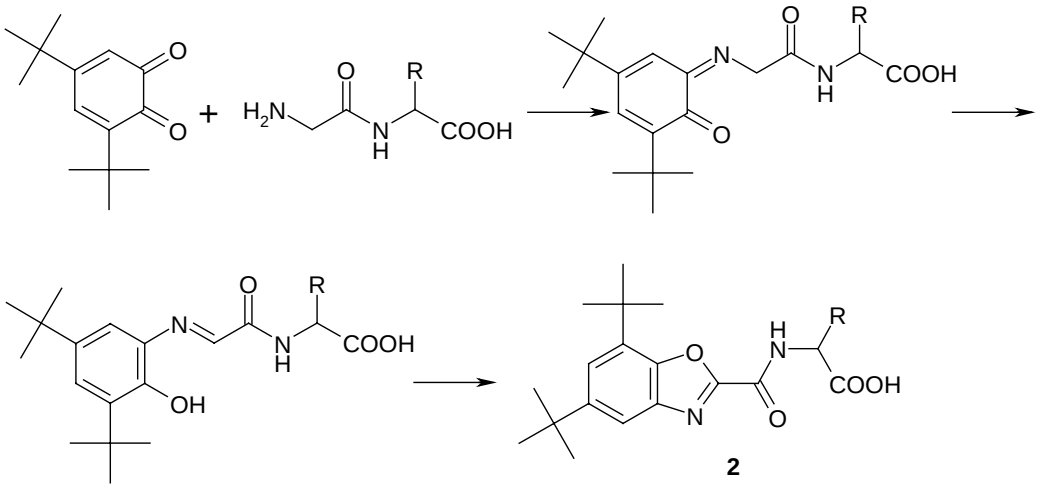
Phenylserine (R = H) produces the corresponding benzoxazole **2l** as the product, while phenylserine methyl ester (R = CH_3) reacts in a different way. The latter produces benzoxazole **2m** in a reaction involving C-C bond cleavage, in accordance with our previous results with 2-aminoethanol derivatives carrying benzylic hydroxyls [12] (Scheme 3).

Scheme 3



Dipeptides with N-terminal glycine (Gly-Gly, Gy-Leu, Gly-Tyr) afforded benzoxazole derivatives **2n**, **2o**, **2p** (Scheme 4).

Scheme 4



2

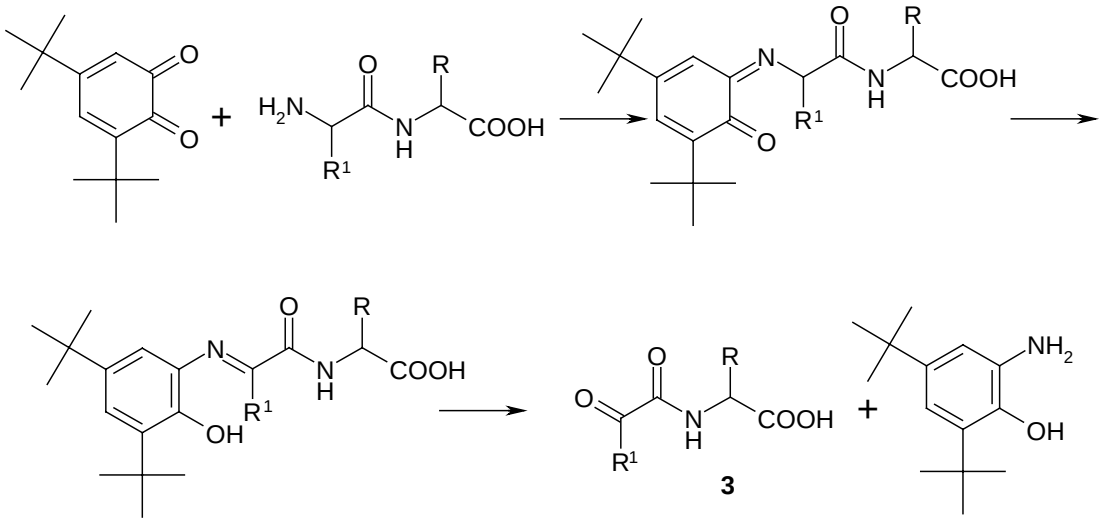
2n R = H

2o R = $CH_2CH(CH_3)_2$

2p R = $CH_2C_6H_4$(4-OH)

As expected, dipeptides carrying at the N-terminal an amino acid other than glycine (Ala-Gly, Phe-Phe and Leu-Gly) underwent Correy´s oxidative deamination [13] with formation of ketoacylamino acids **3a**, **3b**, **3c**, (Scheme 5). Two of them were characterized by their 2,4-dinitrophenylhydrazones **4a**, **4b** (Scheme 6).

Scheme 5



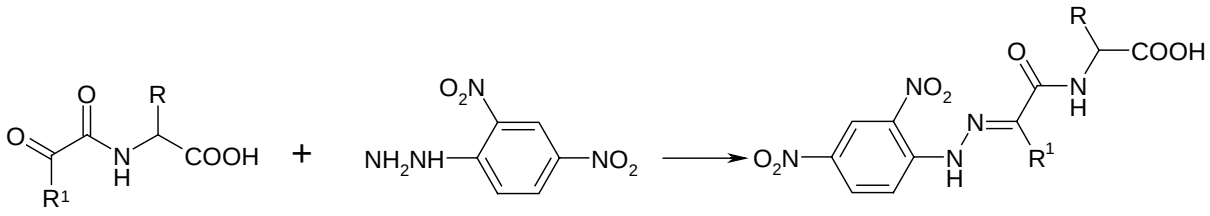
3

3a R = H $R^1 = CH_3$

3b R = $CH_2C_6H_5$ $R^1 = CH_2C_6H_5$

3c R = H $R^1 = CH_2CH(CH_3)_2$

Scheme 6



4

4a R = H $R^1 = CH_3$

4b R = $CH_2C_6H_5$ $R^1 = CH_2C_6H_5$

Biological activity

Antimycobacterial activity was evaluated against a set of four mycobacterium strains: *Mycobacterium tuberculosis* CNCTC My 331/88, *Mycobacterium kansasii* CNCTC My 235/80, *M. kansasii* 6509/96 and *Mycobacterium avium* CNCTC My 330/88 using the micro method for determination of the minimum inhibitory concentration (MIC), the lowest concentration of a substance, at which the inhibition of growth of mycobacteria occurred. The following concentrations were used: 1000, 500, 250, 125, 62.5, 32, 16, 8 and 4 μmol·L^{-1}. Results of the tests are shown in Table 1. The tested compounds have shown promising activity, ranging from 16 μmol·L^{-1} to 500 μmol·L^{-1}. The most active was the benzoxazole 4-(5,7-di-*tert*-butylbenzoxazole-2-yl-methyl)-phenol (**2h**).

Table 1. *In vitro* antimycobacterial activity of compounds expressed as MIC (μmol·L^{-1})

Compound	Strains									
	Mycobacterium kansasii 6 509/96 conc. 10^{-4}			*Mycobacterium kansasii* My 235/80 conc. 10^{-4}			*Mycobacterium avium* My 330/88 conc. 10^{-5}		*Mycobacterium tuberculosis* My 331/88 conc. 10^{-3}	
Time	7d	14 d	21 d	7d	14 d	21 d	7d	14 d	14 d	21 d
2e	500	1000	1000	500	125	250	1000	>1000	500	500
2h	16	32	62.5	32	62.5	125	250	500	125	125
2i	62.5	125	125	62.5	125	500	250	250	250	250
2j	62.5	62.5	62.5	62.5	125	125	125	250	125	125
2n	250	500	500	250	500	500	1000	1000	500	500
2o	125	500	500	250	500	1000	1000	500	250	250
2p	500	1000	1000	1000	1000	1000	1000	>1000	500	1000
INH	2	2	4	>250	>250	>250	>250	>250	0.5[a]	1.0[a]

[a] conc. 10^{-4}

The *in vitro* antifungal activity was tested against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon beigelii* 1188 (TB), *Trichophyton mentagrophytes* 445 (TM), *Aspergillus fumigatus* 231 (AF) and *Absidia corymbifera* 272 (AC) by using the microdilution broth test [14]. All strains, except CA were clinical

isolates, identified by conventional morphological and biochemical methods. All the studied compounds were nearly inactive in concentrations of less than 500 μmol·L^{-1} MIC against all strains, with the exception of compound **2j**, which showed 62.5 μmol·L^{-1} MIC against CA and 125 μmol·L^{-1} MIC against AC.

Conclusions

Preliminary biological evaluation has shown that a number of our newly synthesised highly lipophilic benzoxazole derivatives possess antimycobacterial activity. This included activity against nontuberculous mycobacteria such as *Mycobacterium kansasii* isolated from a clinical isolate and *Mycobacterium avium,* where isoniazid is inactive. The possible improvement of the antituberculotic properties of these structures, through the modulation of the benzoxazole substitution and/or further functionalisation warrants further investigation. Antifungal testing against selected strains has not shown any significant activity.

Acknowledgements

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Experimental

General

Chemicals were purchased from Aldrich. Melting points (uncorrected) were determined on a Kofler block. Elemental analyses were performed on CHNS-O CE instrument (FISONS EA 1110) and were within ±0.4 % of calculated values. UV spectra were measured on Polarimeter ADP 220 (BS Bellingham Stanley Ltd.). IR spectra were recorded on Nicolet Impact 400 spectrometer in KBr pellets, Nujol mulls or $CHCl_3$ solutions. NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solutions at ambient temperature in a Varian Mercury-VxBB 300 spectrometer operating at 300 MHz. The chemical shifts δ are given in ppm related to tetramethylsilane (TMS) as internal standard. The coupling constants (*J*) are reported in Hz. Log P was calculated by using ChemDraw Ultra version 6.0.1. The reactions were monitored and the purity of the products was checked by TLC (Silufol UV 254, Kavalier Votice, Czech Republic and Merck TLC plates silica gel 60 F_{254}, aluminium back) usually with ethyl acetate – light petroleum ether (1:9) or ethyl acetate-toluene (1:4) as eluents. The plates were visualized using UV light, iodine fumes, or carbonyl group detection by reaction with 2,4-dinitrophenylhydrazine. Preparative TLC was carried out on silica gel 60 F_{254} (0.015 –0.040 mm, Merck). Silica gel 60 (0.015-0.040 mm, Merck) was used for column chromatography.

General synthesis procedure

Amino acid (dipeptide) (1 mmol) and DTBBQ (1 mmol) were dissolved in ethanol (50 mL, 60 - 96%) and heated for 5 hrs at 50 °C. The solvent was removed and the residue separated by column chromatography or repeatedly by preparative TLC on 20 x 20 cm plates developed with mixtures of

ethyl acetate-petroleum ether (EA-PE), ethyl acetate- toluene (EA-Tol) or petroleum ether-diethyl ether (PE-E) in the appropriate ratios. Purity of isolated layers was checked by TLC using a minimum of two types of developing solvents.

5,7-Di-tert-butylbenzoxazole (**2a**). $C_{15}H_{21}NO$ = 231.33; Yield 45 %; m.p. 50 °C (lit [15] m.p. 53-55 ºC); prep. TLC (EA-PE 1:9) R_f = 0.32, TLC ($CHCl_3$-MeOH 9:1) R_f = 0.71; (MeOH-H_2O) value?; IR ($CHCl_3$) ν_{max} 2961, 2908, 2870 (C-H), 1616, 1520 (C=N), 1482 (C=C), 1392, 1365 (CH_3) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.08 (s, 1H, Ar); 7.66 (d, 1H, *J*=1.65 Hz, Ar), 7.34 (d, 1H, *J*=1.65 Hz, Ar); 1.49 (s, 9H, CH_3), 1.39 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 152.02, 147.70, 140.27, 134.09, 119.86, 114.56, 35.02, 34.37, 31.80, 29.83; UV (EtOH) 211, 236, 275 nm; cLogP 5.068.

5,7-Di-tert-butyl-2-methylbenzoxazole (**2b**). $C_{16}H_{23}NO$ = 245.18; Yield 72 %; m.p. 58-61 °C; prep. TLC PE-E (1:1) R_f = 0.64; TLC CH_2Cl_2 R_f = 0.13, $CHCl_3$-MeOH (9:1) R_f = 0.75, EA-PE (9:1) R_f = 0.28; IR (KBr) ν_{max} 2955, 2906, 2869 (C-H), 1608, 1582 (C=N), 1483, 1466 (C=C), 1394, 1363 (CH_3), 873, 843 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.51 (d, 1H, J=1.92 Hz, Ar), 7.23 (d, 1H, J=1.92 Hz, Ar), 2.63 (s, 3H, CH_3), 1.47 (s, 9H, CH_3), 1.38 (s. 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 163.10, 147.11, 141.67, 133.35, 118.66, 113.59, 34.96, 34.36, 31.82, 29.88, 14.63; M^+ = 245 m/e; UV(EtOH) 208, 236, 274 nm; cLogP 5.336.

5,7-Di-tert-butyl-2-phenylbenzoxazole (**2c**)**.** $C_{21}H_{25}NO$ = 307.43; Yield 68 %; m.p. 59-60 °C (EtOH), lit [16] m.p. 60-61 °C (MeOH); prep. TLC EA-PE (1:9) R_f = 0.52; TLC EA-Tol (1:4) R_f = 0.80; IR (KBr) ν_{max} 2957, 2907, 2868 (C-H), 1624, 1557 (C=N), 1482 (C=C), 1391, 1362 (CH_3), 863, 706 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.30–8.23 (m, 2H, Ar); 7.67 (d, 1H, *J* = 1.79 Hz, Ar); 7.57–7.50 (m, 3H, Ar); 7.32 (d, 1H, *J*=1.79 Hz, Ar); 1.56 (s, 9H, CH_3); 1.41 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 162.45, 147.72, 146.91, 142.28, 133.70, 131.17, 128.86, 127.51, 127.38, 119.54, 114.20, 35.07, 34.46, 31.81, 30.01; UV (EtOH) 207, 239, 296 nm; cLogP 7.165.

5,7-Di-tert-butyl-2-benzylbenzoxazole (**2d**). $C_{22}H_{27}NO$ = 321.46; Yield 73 %; oil; prep. TLC EA-PE (1:9) R_f = 0.18; IR (Nujol) ν_{max} 1605, 1575 (C=N), 1582 (C=C), 1391, 1360 (CH_3), 868, 849, 722 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.55 (d, 1H, *J*=1.92 Hz, Ar), 7.42-7.27 (m, 5H, Ar), 7.25 (d, 1H, *J*=1.93 Hz, Ar), 4.27 (s, 2H, CH_2), 1.45 (s, 9H, SH_3), 1.36 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 164.65, 147.32, 147.21, 142.86, 135.28, 133.58, 128.91, 127.13, 119.00, 113.92, 35.29, 34.35, 31.79, 31.57, 29.60; UV (EtOH) 211, 238, 276 nm; cLogP 6.904.

5,7-Di-tert-butyl-2-(1-methyl)ethylbenzoxazole (**2e**). $C_{18}H_{27}NO$ = 273.21; Yield 52.6 %; oil; prep. TLC EA-PE (1:9) R_f = 0.55, then PE-E (1:1) R_f = 088; IR (KBr) ν_{max} 2958, 2907, 2871 (C-H), 1608, 1574 (C=N), 1482 (C=C), 1390, 1363 (CH_3), 869, 837 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.58 (d, 1H, J=1.79 Hz, Ar), 7.25 (d, 1H, J=1.79 Hz, Ar), 3.34-3.18 (m, 1H, CH), 1.48 (s, 9H, CH_3), 1.46 (d, 6H, J=7.69, CH_3), 1.37 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 170.75, 147.24, 146.86, 141.02, 133.48, 118.75, 113.75, 34.99, 34.36, 31.82, 29.87, 28.79, 20.35; UV (EtOH) 208, 236, 276 nm; cLogP 6.264.

5,7-Di-tert-butyl-2-(2-methyl)propylbenzoxazole (**2f**)**.** $C_{19}H_{29}NO$ = 287.44; Yield 34 %; oil; prep. TLC EA-PE (1:9) R_f = 0.87; IR ($CHCl_3$) ν_{max} 2959, 2908, 2871 (C-H), 1607, 1575 (C=N), 1482 (C=C),

1392, 1364 (CH_3), 868 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.54 (d, 1H, *J*=1.79 Hz, Ar), 7.24 (d, 1H, *J*=1.79 Hz, Ar), 2.81 (d, 2H, *J*=7.14 Hz, CH_2), 2.38-2.19 (m, 1H, CH), 1.48 (s, 9H, CH_3), 1.37 (s, 9H, CH_3), 1.05 (d, 6H, *J*=6.6 Hz, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.95, 147.07, 141.52, 133.42, 118.64, 113.72, 37.58, 34.98, 34.35, 31.83, 29.87, 27.56, 22.42; UV (EtOH) 212, 237, 275 nm; cLogP 6.793.

5,7-Di-tert-butyl-2-(2-methylthioethyl)-benzoxazole (**2g**)**.** $C_{18}H_{27}NOS$ = 305.48; Yield 67.3 %; m.p. 95 °C; column chromatography EA-PE (0.5:9.5), then prep. TLC EA-PE (2:8) R_f = 0.45; IR ($CHCl_3$) ν_{max} 2966, 2921, 2910, 2871 (C-H), 1606, 1574 (C=N), 1482 (C=C), 1393, 1365 (CH_3) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.55 (d, 1H, *J*=1.92 Hz, Ar), 7.25 (d, 1H, *J*=1.92 Hz, Ar), 3.28-3.20 (m, 2H, CH_2), 3.07-2.99 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.47 (s, 9H, CH_3), 1.36 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 142.13, 142.07, 140.54, 135.59, 115.95, 110.36, 34.89, 34.40, 31.63, 29.74, 15.60; UV (EtOH) 213, 238, 275 nm; cLogP 5.634.

4-(5,7-Di-tert-butylbenzoxazol-2-yl-methyl)-phenol (**2h**). $C_{22}H_{27}NO_2$ = 337.46; Yield 35.7 %; m.p. 121-123 °C (EtOH/H_2O); TLC EA-PE (1:9) R_f = 0.64; IR (KBr) ν_{max} 3440 (O-H), 2959, 2906, 2869 (C-H), 1615, 1517(C=N), 1479 (C=C), 1392, 1363 (CH_3), 834, (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.53 (d, 1H, *J*=1.92 Hz, Ar), 7.26 (d, 1H, *J*=1.92 Hz, Ar), 7.17-7.10 (m AA´BB´, 2H, Ar), 6.73-6.67 (m, AA´BB´, 2H, Ar), 4.18 (s, 2H, CH_2), 1.47 (s, 9H, CH_3), 1.34 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.72, 155.51, 147.64, 147.01, 140.84, 133.73, 130.05, 126.13, 119.20, 115.91, 113.58, 35.01, 34.38, 34.35, 31.47, 29.89; UV (EtOH) 217, 279, 328 nm; cLogP 6.237.

5,7-Di-tert-butyl-2-[1H-indol-3-yl(methyl)]-benzoxazole (**2i**). $C_{24}H_{28}N_2O$ = 360.49; Yield 82%; M.p.: 159-161 °C (EtOH/H_2O); column chromatography PE-CH_2Cl_2 (2:8), then EA-PE (1:9) R_f = 0.57; IR (KBr) ν_{max} 3390 (N-H), 2963, 2906, 2870 (C-H), 1617, 1575 (C=N), 1481, 1458 (C=C), 1391, 1363 (CH_3), 871, 845 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.28 (bs, 1H, NH), 7.75 (d, 1H, J=7.7 Hz, Ar), 7.55 (d, 1H, J=1.9 Hz, Ar), 7.37-7.32 (m, 1H, Ar), 7.24 (d, 1H, J=1.9 Hz, Ar), 7.23-7.10 (m, 3H, Ar), 4.44 (s, 2H, CH_2), 1.46 (s, 9H, CH_3), 1.35 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.21, 147.27, 147.09, 141.30, 136.16, 133.56, 126.99, 122.89, 122.23, 119.64, 118.92, 113.81, 111.17, 109.48, 34.98, 34.35, 31.80, 29.90, 25.38; UV (EtOH) 223, 242, 276 nm; cLogP 6.894.

5,7-Di-tert-butylbenzoxazole-2-carboxamide (**2j**)**.** $C_{16}H_{22}N_2O_2$= 274.17; Yield 61 %; m.p. 193-194 °C (EtOH); column chromatography in Tol, then EA-Tol (1:4), TLC R_f = 0.2; TLC EA-PE (1:9) R_f = 0.52; IR (KBr) ν_{max} 2961, 2907, 2871(C-H), 1701(C=N), 1619, 1600 (C=N), 1546 (C=N), 1483 (C=C), 1395, 1364 (CH_3), 870, 841 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.63 (d, 1H, J=1.64 Hz, Ar), 7.44 (d, 1H, *J*=1.64 Hz, Ar), 7.25 (bs, 1H, NH_2), 6.42 (bs, 1H, NH_2), 1.51 (s, 9H, CH_3), 1.38 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 157.70, 154.50, 148.96, 140.51, 135.20, 122.11, 115.04, 35.150, 34.57, 31.68, 29.92; UV (EtOH) 213, 231, 274 nm; cLogP 4.566.

1,3-Bis(5,7-di-tert-butylbenzoxazol-2-yl)propane (**2k**). $C_{33}H_{46}N_2O_2$=502,73; Yield 38 %; m.p. 155-157 °C (EA-PE); prep. TLC EA-PE (1:9) R_f = 0.68; TLC Tol-EA (4:1) R_f = 0.83; IR (KBr) ν_{max} 2957, 2906, 2869 (C-H), 1607, 1574 (C=N), 1482 (C=C), 1403, 1363 (CH_3), 869, 769 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.55 (d, 2H, *J*=2.29 Hz, Ar), 7.25 (d, 2H, *J*=2.29 Hz, Ar), 3.13 (t, 4H, *J*=7.41 Hz, CH_2), 2.56-2.44 (m, 2H, CH_2), 1.46 (s, 18H, CH_3), 1.37 (s, 18H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 165.38, 147.25,

147.03, 141.44, 133.52, 118.88, 113.80, 34.99, 34.36, 31.82, 29.90, 27.88, 23.93; UV (EtOH) 216, 239, 275 nm; cLogP 9.978.

(5,7-Di-tert-butylbenzoxazol-2-yl)phenylmethanol (**2l**). $C_{22}H_{27}NO_2$ = 337.20; Yield 33 %; m.p. 112 – 114 °C; column chromatography in CH_2Cl_2 followed by MeOH; TLC $CHCl_3$-MeOH (9:1) R_f = 0.65; IR (KBr) ν_{max} 2961, 2907, 2869 (C-H), 1624, 1570 (C=N), 1482 (C=C), 1403, 1364 (CH_3), 869, 855 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.57-7.52 (m, 3H Ar), 7.42-7.28 (m, 3H Ar), 7.28-7.25 (m, 1H Ar), 6.03 (d, 1H, *J*=5.2 Hz, CH), 3.75 (d, 1H, *J*=5.5 Hz, OH); 1.41 (s, 9H, CH_3), 1.36 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 166.08, 147.78, 147.29, 140.48, 139.18, 133.98, 128.66, 128.56, 126.61, 119.60, 114.21, 70.47, 35.04, 34.34, 31.77, 29.83; M^+ = 337 m/e; UV (EtOH) 209, 242, 277 nm; cLogP 4.029.

5,7-Di-tert-butylbezoxazol-2-methylcarbonate (**2m**). $C_{17}H_{23}NO_3$ = 289.37; Yield 17 %; oil; preparative TLC $CHCl_3$-MeOH (9:1) R_f = 0.78; IR ($CHCl_3$) ν_{max} 2967, 2908, 2872 (C-H), 1746 (CO-ester), 1616, 1544 (C=N), 1483 (C=C), 1365 (CH_3) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.69 (d, 1H, *J*=1.92 Hz, Ar); 7.46 (d, 1H, *J*=1.92 Hz, Ar); 4.07 (s, 3H, CH_3); 1.51 (s, 9H, CH_3); 1.38 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 157.07, 152.19, 149.10, 147.42, 140.85, 135.01, 122.66, 115.71, 53.43, 35.14, 34.53, 31.64, 29.85; UV (EtOH) 213, 233, 275 nm; cLogP 4.789.

[(5,7-di-tert-butylbenzoxazole-2-carbonyl)-amino]-acetic acid (**2n**)**.** $C_{18}H_{24}N_2O_4$ = 332.39; Yield 80 %; m.p. 182-183 °C; isolation by extraction with PE. Insoluble crystalline part recrystallized from EA-PE; TLC $CHCl_3$-MeOH (9:1) R_f = 0.26, 1-butanol-formic acid-water (75:15:10) R_f = 0.87; IR (KBr) ν_{max} 3410 (O-H), 2961, 2908, 2872 (C-H), 1740 (COOH), 1694 ($CONH_2$), 1618, 1559 (C=N), 1482 (C=C), 1391, 1365 (CH_3), 869, 845 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.27 (t, 1H, *J*=5.63 Hz, NH), 7.61 (d, 1H, *J*=1.79 Hz, Ar), 7.43 (d, 1H, *J*=1.79 Hz, Ar), 4.37 (d, 2H, *J*=5.77 Hz, CH_2), 1.50 (s, 9H, CH_3), 1.37 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 172.73, 156.15, 154.47, 149.23, 147.37, 139.73, 135.21, 122.27, 114.66, 41.27, 35.12, 34.46, 31.60, 29.77; UV (EtOH) 217, 231, 264 nm; cLogP 4.673.

2-[(5,7-Di-tert-butylbenzoxazole-2-carbonyl)-amino]-4-methylpentanoic acid (**2o**). $C_{22}H_{32}N_2O_4$ = 388.50; Yield 41.3 %; m.p. 183-184 °C; isolation by extraction with PE. Insoluble crystalline part recrystallized from EA-PE; TLC $CHCl_3$-MeOH (9:1) R_f=0.32, 1-butanol-formic acid-water (75:15:10) R_f = 0.62; $[\alpha]_D^{25}$ = 39.32 °(c = 0.9; EA); IR (KBr) ν_{max} 3408 (O-H), 2961, 2907, 2869 (C-H), 1725 (COOH), 1685 ($CONH_2$), 1618, 1557 (C=N), 1482 (C=C), 1391, 1364 (CH_3), 869 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.85 (d, 1H, *J*=8.52 Hz, NH), 7.62 (d, 1H, *J*=1.79 Hz, Ar), 7.42 (d, 1H, *J*=1.79 Hz, Ar), 6.72 (bs, 1H, COOH), 4.92-4.82 (m, 1H, CH), 1.91-1.72 (m, 3H, CH, CH_2), 1.50 (s, 9H, CH_3), 1.37 (s, 9H, CH_3), 1.00 (d overlapped, 3H, *J*=6.05 Hz, CH_3), 0.99 (d, overlapped, 3H, *J*=6.05 Hz, CH_3); ^{13}C-NMR ($CDCl_3$) δ 176.35, 155.76, 154.62, 149.06, 147.61, 140.10, 135.23, 122.09, 114.83, 51.04, 41.08, 35.15, 34.53, 31.67, 29.89, 24.92, 21.68; UV (EtOH) 212, 233, 279 nm; cLogP 6.439.

2-[(5,7-Di-tert-butylbenzoxazole-2-carbonyl)-amino]-3-(4-hydroxyphenyl)propionic acid (**2p**). $C_{25}H_{30}N_2O_5$ = 438.52; Yield 64 %; m.p.: 126 -128 °C; isolation by extraction with PE. Insoluble crystalline part recrystallized from E-PE; $[\alpha]_D^{25}$ = 36.58 °(c=0.8; EA); TLC EA-Tol (1:4) R_f = 0.1, $CHCl_3$-MeOH (7:3) R_f = 0.85; IR (KBr) ν_{max} 3405 (O-H), 2962, 2909, 2871(C-H), 1724 (COOH), 1678 ($CONH_2$), 1616, 1558 (C=N), 1483 (C=C), 1392 , 1365 (CH_3), 877 ,837(Ar-H) cm^{-1}; ^{1}H-NMR

($CDCl_3$) δ 9.20 (bs, 1H, OH), 9.12 (d, 1H, J=8.24 Hz), 7.66 (d, 1H, *J*=1.64 Hz, Ar), 7.41 (d, 1H, *J*=1.92 Hz, Ar), 7.11-7.04 (m AA´BB´, 2H, Ar), 6.67-6.60 (m AA´BB´, 2H, Ar), 4.67-4.52 (m, 1H, CH), 3.14-3.01 (m, 2H, CH_2), 1.44 (s, 9H, CH_3), 1.34 (s, 9H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 172.42, 156.16, 155.38, 155.13, 148.63, 146.75, 140.44, 134.58, 130.29, 127.80, 121.59, 115.26, 115.16, 54.50, 40.54, 35.23, 34.36, 31.67, 29.83; UV (EtOH) 209, 242, 277 nm; cLogP 5.733.

(2-Oxo-propionylamino)acetic acid (**3a**). $C_5H_7NO_4$ = 145.11; Yield 65 %. M.p.: 88 °C (EA-PE), lit. [17] m.p. 90 °C; TLC 1-butanol-formic acid-water (75:15:10) R_f = 0.63; IR (KBr) ν_{max} 3283 (N-H), 1734 (CO-COOH), 1683 (CO), 1663 (amide-I), 1538 (amide II), 1412, 161 (CH_3), 1183 (C-O) cm^{-1}; ^{1}H NMR (D_2O) δ 4.1 (s, 2H, CH_2), 2.2 (s, 3H, CH_3); ^{13}C-NMR (D_2O) δ 200.00, 177.93, 165.10, 43.61, 26.95

2-(2-Oxo-3-phenyl-propionylamino)-3-pheyl-propionic acid (**3b**). $C_{18}H_{17}NO_4$ = 311.33; Compound was isolated as **4b**

4-Methyl-2-oxo-pentanoylamino)-acetic acid (**3c**). $C_8H_{13}NO_4$ = 187.19; Yield 20 %; oil, TLC $CHCl_3$-MeOH (9:1) R_f=0,21; IR ($CHCl_3$) ν_{max} 3404 (O-H), 2963, 2935, 2874 (C-H), 1732 (COOH), 1689 ($CONH_2$), 1526, 1467, 1438, 1370 cm^{-1}; ^{1}H-NMR (DMSO) δ 7.51 (bs, 1H, NH), 7.13 (d, 2H, *J*=5.49, CH_2), 2.80 (d, 2H, *J*=6.87 Hz, CH_2) 2,35-1.98 (m, 1H, CH), 0.95 (d, 6H, *J*=6.6 Hz, CH_3)

{2-[(2,4-dinitrophenyl)-hydrazono]-propionylamino}acetic acid (**4a**) $C_{11}H_{11}N_5O_7$ = 325.23; Yield 45 %; m.p.: 245 – 246 °C (EtOH/H_2O), lit. [18] m.p.: 245-246 °C; IR (KBr) ν_{max} 3314 (N-H); 3108, 3095 (=C-H), 1737 (-CO-C=N); 1654 ($CONH_2$); 1619, 1341 (NO_2); 848, 833(Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.22 (bs, 1H, OH), 9.18 (d, 1H, *J*=2.47 Hz, H3´), 8.44 (ddd, 1H, *J*=9.48 Hz, *J*=2.47 Hz, *J*=0.55 Hz, H5´), 8.00 (d, 1H, *J*=9.48 Hz, H6´), 7.47 (t, 1H, *J*=4.94 Hz, NH), 4.19 (d, 2H, *J*=5.22 Hz, CH_2), 2.32 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ 169.76, 163.08, 146.53, 143.92, 139.60, 139.60, 123.28, 116.62, 61.84, 41.52, 14.16; UV (EtOH) 207, 264, 351 nm.

2{2-[(2,4-dinitrophenyl)-hydrazono]-3-phenyl-propionylamino}-3-phenylpropionic acid (**4b**) $C_{24}H_{21}N_5O_7$ = 491.45; Yield 35 %; m.p. 131-133 °C (EtOH); IR (KBr) ν_{max} 3420 (N-H); 1736 (-CO-C=N); 1671 ($CONH_2$); 1618, 1339 (NO_2); 742, 702 (Ar-H) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 11.24 (bs, 1H, OH), 9.10 (d, 1H, *J*=2.48 Hz, H3´), 8.33 (dd, 1H, *J*=9.62 Hz, J=2.48, H5´), 7.67 (d, 1H, *J*=9.62 Hz, H6´), 7.57-7.29 (m, 10H), 5.06-4.97 (m, 1H, CH), 4.13 (d, 2H, CH_2), 3.28 (d, 2H, *J*=5.49 Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ 14.18, 30.67, 37.85, 61.84, 116.78, 123.10, 127.31, 127.49, 128.69, 129.23, 129.48, 130.07, 131.09, 133.18, 135.65, 139.63, 143.75, 147.76, 162.86, 171.26; UV (EtOH) 210, 253, 352 nm.

Antimycobacterial testing

All strains were obtained from the Czech National Collection of Type Cultures (CNCTC) with exception of *M. kansasii* 6509/96, which was a clinical isolate. The antimycobacterial activities of the compounds were determined in the Šula semisyntetic medium (SEVAC, Prague). The compounds

were added in form of a solution in dimethyl sulphoxide/water (10 % maximum of DMSO) to the medium. MICs were determined after incubation at 37 °C for 7, 14 and 21 days.

References

1. Temiz, O.; Oren, I.; Sener, E.; Yalcin, I.; Ucartürk, N. *Il Farmaco* **1998**, *53* , 337-341.
2. Oren, I.; Temiz, O.; Yalcin, I.; Sener, E. A.; Altanlar, N. *Eur. J. Pharm. Sci* **1998**, *7*, 153-160.
3. Kumar, D.; Jacob, M. R.; Reynolds, M. B.; Kerwin, S. M. *Bioorg. Med. Chem.* **2002**, *10,* 3997-4004.
4. Kim, J. S.; Sun, Q.; Gatto, B.; Yu, Ch.; Liu, A.; Liu, L. F.; LaVoie, E. *J. Bioorg. Med. Chem.* **1996**, *4*, 621-630.
5. Hoffman, J. M.; Smith, A. M.; Rooney, C. S.; Fisher, T. E.; Wai, J. S.; Thomas, C. M.; Bamberger, D. L.; Barne, J. L.; William, T. M.; Jobes, H. H.; Olson, B. D.; O´Brien, J. A.; Goldman, M. E.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Emini, E. A.; Anderson, P. S. *J. Med. Chem*. **1993**, *36*, 953-966.
6. Perrin, L.; Rakik, A.; Yearly, S.; Baumberger, C.; Kinloch-deLoies, S.; Pechiere, M.; Hirschel, B. *AIDS* **1996,** 10, 1233-1239.
7. Holler, M. G.; Campo, L. F.; Brandelli, A.; Stefani, V. *J. Photochem. Photobiol. A: Chemistry* **2002**, 149, 217-225.
8. Laber, B.; Usunow, G.; Wiecko, E.; Franke, W.; Franke, H.; Köhn, A. *Pesticide Biochem. Physiol* **1999**, *63*, 173-184.
9. Dunwell, D. W.; Evans, D. *J. Med. Chem.* **1977**, *20*, 797-801.
10. Vinsova J., Horak V. *Chem. Listy* **2001**, *97*, 1157-1167.
11. Voleva, V. B.; Belostotskaya, I. S.; Bundel, A. Yu.; Komissarova, N. L.; Erskov, V. V. *Russ. Chem. Bull.* **1997**, *46,* 385-386.
12. Horak, V.; Mermersky, Y.; Guirguis, D. B. *Collect. Czech Chem. Commun.* **1994**, *59*, 227-230.
13. Corey, E. J.; Achiwa, K. *J. Am. Chem. Soc.* **1969**, *91*, 1429-1432.
14. National Committee for Clinical Laboratory Standards: *Reference method for broth dilution antifungal susceptibility testing of yeast: Approved Standard, NCCLS document, M27-A*; NCCLS: Villanova, PA, **1997**.
15. Haeussler, H.; Jadamus, H. *Chem. Ber.* **1964**, *97*, 3051-3055.
16. Gilchrist, T.L.; Harris, C. J.; King, F. D.; Peek, M. E.; Rees, Ch. W. *J. Chem. Soc. Perkin Trans 1* **1988**, 2169-2174.
17. Wieland, T.; Klu, Hi Shin, Heinke, B. *Chem. Ber.* **1958**, *91*, 483-487.
18. Errera, M.; Greenstein, J. P. *Arch. Biochem*. **1947**, *14*, 477-478.

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