Polish Journal of Microbiology
2016, Vol. 65, No 3, 359–364

SHORT COMMUNICATION

In Search of the Antimicrobial Potential of Benzimidazole Derivatives

MONIKA JANECZKO[1], ZYGMUNT KAZIMIERCZUK[2], ANDRZEJ ORZESZKO[2], ANDRZEJ NIEWIADOMY[3], EWA KRÓL[4], RYSZARD SZYSZKA[1] and MACIEJ MASŁYK[1]*

[1]Department of Molecular Biology, Institute of Biotechnology, The John Paul II Catholic University of Lublin, Lublin, Poland
[2]Institute of Chemistry, Warsaw University of Life Sciences, Warsaw, Poland
[3]Department of Chemistry, University of Life Sciences, Lublin, Poland
[4]Department of Phytopatology and Mycology, Faculty of Horticulture and Landscape Architecture, University of Life Sciences, Lublin, Poland

Submitted 16 December 2014, revised 1 June 2015, accepted 11 February 2016

Abstract

A broad series of 4,5,6,7-tetrahalogenated benzimidazoles and 4-(1*H*-benzimidazol-2-yl)-benzene-1,3-diol derivatives was tested against selected bacteria and fungi. For this study three plant pathogens *Colletotrichum* sp., *Fusarium* sp., and *Sclerotinia* sp., as well as *Staphylococcus* sp., *Enterococcus* sp., *Escherichia* sp., *Enterobacter* sp., *Klebsiella* spp. , and *Candida* spp. as human pathogens were used. MIC values and/or area of growth reduction method were applied in order to compare the activity of the synthesized compounds. From the presented set of 22 compounds, only 8, 16, 18 and 19 showed moderate to good inhibition against bacterial strains. Against *Candida* strains only compound 19 with three hydroxyl substituted benzene moiety presented high inhibition at nystatin level or lower.

Key words: antibacterial activity, antifungal activity, benzimidazole

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications (Bansal and Silakari, 2012). This ring system is present in numerous antiparasitic, fungicidal anthelmintic, anti-inflamatory and antiviral drugs (Pedini *et al.*, 1994; Martin, 1997; Zacny *et al.*, 1999; Gaba *et al.*, 2014). Some benzimidazoles show also cytotoxicity against diverse cancer cell lines (Horton *et al.*, 2003; Padmavathi *et al.*, 2008; Karpińska *et al.*, 2012). Recently, we found that polyhalogenated benzimidazoles are potent inhibitors of casein kinase 2 (CK2), probably the most pleiotropic protein kinase in more than 300 known eukaryotic organisms (Pagano *et al.*, 2004; Gianoncelli *et al.*, 2009; Janeczko *et al.*, 2012). Interestingly, prokaryotes do not code for CK2 genes. The antimicrobial activity of benzimidazole derivatives is frequently studied with various microrganisms. Their antimycobacterial, antiprotozoal and antibacterial activity has been observed for variously modified benzimidazole derivatives (Andrzejewska *et al.*, 2004; Kazimierczuk *et al.*, 2005; Navarrete-Vázquez *et al.*, 2006; Laudy *et al.*, 2012). In addition to their antibacterial activity, benzimidazole derivatives possess antifungal activity. Benomyl, thiabendazole and thiophnate methyl are some main examples of this fungicide class. They are also used for the prevention of post-harvest rots and as soil-drench treatments (Kaplancikli *et al.*, 2004).

The major objective of the present study was examination of the antibacterial activity of established CK2 inhibitors possessing 4,5,6,7-tetrabromo- or 4,5,6,7-tetraiodobenzimidazole as a fragment of active structure (compounds 1–16) and with benzene-1,3-diole and benzene-1,2,3-triole substituted at the 2 position of the heterocyclic ring showing anticancer activity (compounds 17–22). Also, we present here the results of antifungal activity of the above mentioned compounds against three *Candida* species obtained as clinic isolates and three plant fungi: *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum* and *Fusarium culmorum*.

The preliminary results presented below indicate that the biological potential of investigated benzimidazole derivatives is not limited to the previously reported features as CK2 inhibitors or anticancer potential.

For this study a series of benzimidazole derivatives were used. Compounds 1–16 were obtained as previously described (Janeczko *et al.*, 2012). 4,5,6,7-Tetrabromo- and 4,5,6,7-tetraiodobenzimidazoles and their respective 2-mercaptoderivatives were alkylated with ω-bro-

* Corresponding author: M. Masłyk, Department of Molecular Biology, Institute of Biotechnology, The John Paul II Catholic University of Lublin, Lublin, Poland; **e-mail: maciekm@kul.pl**

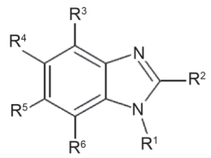
moalkyl esters in the presence of potassium carbonate in aprotic solvents to yield the desired compounds. The respective benzimidazoles containing ester moieties were hydrolyzed in alkaline medium to yield the corresponding carboxyalkyl compounds. The structures of the new compounds were confirmed by ^{1}H NMR and UV spectra and elemental analyses.

Compounds 17–22 were synthesized as previously described (Karpińka *et al.*, 2011; 2012; Los *et al.*, 2012). The compounds were formed by the reaction of *N*-alkyl(aryl)benzene-1,2-diamine derivatives with sulfinylbis[(2,4-dihydroxyphenyl)-methanethione] (STB) or its analogues in methanol under reflux (2.5–3.5 h) in moderate to good yields (63–77%). Structures of benzimidazoles used in this study are shown in Table I. The logP values were determined using the Molinspiration cheminformatics tool (http://www.molinspiration.com/cgi-bin/properties).

Antibacterial activity tests were carried out against the reference strains: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus hominis* (PCM 2651), *Enterococcus faecalis* (PCM 2673), *Escherichia coli* (ATCC 8739), *Enterobacter cloacae* (PCM 2569), *Klebsiella oxytoca* (clinical isolate) and *Klebsiella pneumoniae* ssp. *pneumoniae* (PCM 1). Strains of bacteria were inoculated in Mueller-Hinton broth (Biocorp, Poland) for 24 h before performing the minimal inhibitory concentration (MIC) test, and incubated at 37°C with vigorous shaking (180 rpm). MIC was determined by the microbroth dilution method. Bacterial suspensions in Mueller-Hinton liquid medium at initial inoculums of 5×10^5 colony forming units per ml were added to polystyrene 96-well plates were exposed to the investigated benzimidazoles at adequate concentrations (range: 1–5 mg/ml) for 20 h at 37°C. MIC's were taken as the lowest drug concentration at which observable growth was inhibited. Tetracycline and chloramphenicol were used as reference compounds. Experiments were performed in triplicate.

The fungal strains tested were: 5 strains of *Candida albicans* (clinical isolates), 5 strains of *Candida glabrata* (clinical isolates) and 5 strains of *Candida tropicalis* (clinical isolates) and plant pathogens: *C. gloeosporioides*, *S. sclerotiorum* and *F. culmorum.* All clinical isolates were obtained from Paweł Kozak, Laboratory of Microbiology and Mycobacterium tuberculosis, The

Table I
Chemical structures of benzimidazole derivatives

Compound		MW	Log *P*
1	R^1 = H, R^2 = H, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	433.7	4.13
2	R^1 = CH_2COOH, R^2 = H, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	491.7	3.63
3	R^1 = H, R^2 = H, R^3 = I, R^4 = I, R^5 = I, R^6 = I	621.7	6.24
4	R^1 = CH_2COOH, R^2 = H, R^3 = I, R^4 = I, R^5 = I, R^6 = I	679.8	5.74
5	R^1 = H, R^2 = SCH_2COOH, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	523.8	4.81
6	R^1 = H, R^2 = $S(CH_2)_2COOH$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	537.8	5.11
7	R^1 = H, R^2 = $S(CH_2)_3COOH$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	551.9	5.39
8	R^1 = CH_3, R^2 = SCH_2COOH, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	537.8	5.05
9	R^1 = CH_3, R^2 = $S(CH_2)_2COOH$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	551.9	5.34
10	R^1 = CH_3, R^2 = $S(CH_2)_3COOH$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	551.9	5.62
11	R^1 = H, R^2 = SCH_2COOH, R^3 = I, R^4 = I, R^5 = I, R^6 = I	711.8	6.93
12	R^1 = H, R^2 = $N(CH_3)_2$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	476.8	5.39
13	R^1 = CH_3, R^2 = $N(CH_3)_2$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	490.8	5.63
14	R^1 = $(CH_2)_3COOH$, R^2 = H, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	519.8	4.20
15	R^1 = CH_2COOH, R^2 = $N(CH_3)_2$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = Br	534.8	4.89
16	R^1 = $(CH_2)_3COOH$, R^2 = $N(CH_3)_2$, R^3 = Br, R^4 = Br, R^5 = Br, R^6 = B	562.9	5.47
17	R^1 = H, R^2 = 2,4-di-OH-5-Et-C_6H_2, R^3 = H, R^4 = CH_3, R^5 = CH_3, R^6 = H	282.3	3.96
18	R^1 = H, R^2 = 2,4-di-OH-5-Cl-C_6H_2, R^3 = H, R^4 = CH_3, R^5 = CH_3, R^6 = H	288.7	3.62
19	R^1 = H, R^2 = 2,3,4-tri-OH-C_6H_2, R^3 = H, R^4 = CH_3, R^5 = CH_3, R^6 = H	270.3	2.67
20	R^1 = CH_3, R^2 = 2,4-di-OH-C_6H_3, R^3 = H, R^4 = H, R^5 = H, R^6 = H	240.3	2.32
21	R^1 = C_6H_6, R^2 = 2,4-di-OH-5-Et-C_6H_2, R^3 = H, R^4 = H, R^5 = H, R^6 = H	330.4	4.89
22	R^1 = $(CH_2)_2OH$, R^2 = 2,4-di-OH-C_6H_2, R^3 = H, R^4 = NO_2, R^5 = H, R^6 = H	315.3	1.84

John of God Independent Public Provincial Hospital, Lublin, Poland.

Candida species were cultured in Sabouraud glucose liquid medium (Biocorp, Poland) for 48 h at the temperature 25°C and shaking (130 rpm). Activity of benzimidazoles against yeast was determined by the microbroth dilution method. Microbial cells suspensions at initial inoculums of 3×10^3 colony forming units per ml in Sabouraud glucose broth were exposed to the benzimidazoles at adequate concentrations (range: 0.001–5 mg/ml) for 48 h at 25°C. MIC was the lowest concentration of the compound that inhibited the visible growth of a microorganism after incubation. Nystatin was used as the control drug. Each experiment was replicated thrice.

Cultures of *C. gloeosporioides*, *S. sclerotiorum* and *F. culmorum* were carried out in solid Potato Dextrose Agar medium (Biocorp, Poland) at room temperature for 7 days. Antifungal activity was determined by the method of serial twofold dilutions. The dilution series of the tested compounds, ranging from 0.0005 to 2 mg/ml, were prepared in molten Potato Dextrose Agar medium. Identical mycelial fragments were applied to the Petri plates. The inoculated plates were incubated at room temperature for 7 days. The MIC values were determined as the lowest concentration of the test sample inhibiting visible fungal growth. Metconazole and tebukonazole were used as control compounds. The experiments were carried out in triplicate.

Antibiotic resistance is one of the most important issues not only in microbiology and clinical medicine, but is also a major public health problem. In the last 25 years the incidence of microbial infections has risen in the world, which generally is associated with the formation and expansion of antibiotic resistance in primary sensitive microorganisms. This phenomenon is due to genetic mutations of organisms that produce defense mechanisms to respond to environmental factors against their life processes. One way to counteract these difficulties is the conscious use of the currently available antibiotics. On the other hand, it is necessary to develop and synthesize new compounds, distinctly different from the existing ones, but synthesized on the basis of structural elements with known biological activity. Here we present a set of benzimidazole derivatives possessing antimicrobial potential. All compounds from Table I were tested against a set of bacterial and fungal strains using microbroth dilution method for bacterial and *Candida* strains as well as serial twofold dilutions method for *Colletotrichum*, *Sclerotinia* and *Fusarium* strains.

From the presented set of 22 compounds, only 8, 16, 18 and 19 showed moderate to good inhibition against bacterial strains. The antibacterial screening indicated that among the tested bacterial strains, good inhibitory results were obtained only against *Staphylococcus* species (Table II). This strain is an opportunistic pathogen that forms the natural microflora of the human organism, but under certain conditions can lead to infections. *Staphylococcus* species may occasionally cause generally hospital-acquired infections in patients whose immune system is impaired, for example by chemotherapy or predisposing illness. The activity of our compound against *Staphylococcus* was observed in case of tetrabromoderivatives (7–9 and 16) substituted with bromine atoms at R^3-R^6 positions as well as benzene derivatives (18, 19) substituted with benzene ring at R^2 position. MIC values were found in the range of 0.6–5.0 mg/ml. Other compounds didn't show any influence on the tested strains in the used concentrantion range. Similar results were obtained by Vinodkumar *et al.* (2008) for 2 – (4-phenylethynyl-phenyl)-benzimidazoles and their derivatives. The MIC's for *S. aureus* and *S. typhimurium* in this case were in the range of 0.2–0.5 mg/ml. In the study of Shingalapur *et al.* (2009) the effect of 5 – (nitro/bromo)-styryl-benzimidazole-2 on the growth of *S. aureus*, *E. faecalis*, *E. coli* and *K. pneumoniae* was tested. MIC values obtained (1–4 μg/ml) suggest the positive effect of nitro- and bromo- substituents on antibacterial activity of such compounds. However, the introduction of bromo substituents on the aromatic ring has highly increased the activity compared to the nitro group. Moreover, the growth of *S. aureus* was also effectively inhibited by trichloro-derivatives. 5,6-dichloro-2-amine and 5-chloro-2-4-benzyloxyphenyl benzimidazole gave MIC values equal to 3.12 μg/ml (Tunçbilek *et al.*, 2009).

In recent years, an increase in the incidence of fungal infections is clearly visible. Paradoxically, it is correlated with the progress of the medicine. Particularly affected are people who are undergoing treatment with immunosuppressive antibiotics with a broad spectrum of activity and transplant patients who are in neutropenia. Fungal infections are caused primarily by strains of *Candida*, especially *C. albicans* and *C. tropicalis*, although recently increases the frequency of isolation of species such as *C. parapsilosis*, *C. glabrata* and *Candida krusei.* Thus, the verification of the antifungal activity of the presented compounds was obligatory. For determination of antifungal activity, all compounds were tested against fifteen clinical isolates of *C. albicans*, *C. glabrata* and *C. tropicalis*. Five clinical isolates from the vagina and urine were used in the case of *C. albicans*. Compounds 16, 19 and 21 showed activity below 5 mg/ml in case of at least one isolate (Table II). In the case of *C. glabrata* isolated from urine, sputum and vagina, only compounds 17–20, and 22 showed good inhibition of growth with MIC values below 1 mg/ml (Table II). *C. tropicalis* on the other hand was found to be the most resistant among all tested human pathogens from the derived fungi (Table II). In this case only two

Table II
MIC values (mg/ml) of benzimidazole derivatives against bacterial, Candida, and phytopathogen strains.

Compound	*S. aureus*	*S. epidermidis*	*S. hominis*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*C. albicans*§					*C. glabrata*§					*C. tropicalis*§					*C. gloeospo-rioides*	*F. culmorum*	*S. sclerotio-rum*
								A	B	C	D	E	F	G	H	I	J	K	L	M	N	O			
1	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	0.5	0.25
2	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	2–5	0.5
3	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	1.25	5–10	5–10	5–10	2.5	5–10	5–10	5–10	2.5	0.125	0.25	0.125
4	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	1	1	0.25
5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	0.5	0.25
6	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	0.5	0.5
7	2.5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	1	0.125
8	5	0.6	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	2–5	0.125
9	5	5–10	1.25	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	2–5	0.5
10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2–5	2–5	0.125
11	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2.5	5–10	5–10	1	1	0.5
12	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	1	1	0.5
13	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2–5	2–5	1
14	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2–5	2–5	0.5
15	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2–5	2–5	1
16	1.25	1.25	0,6	5–10	5–10	5–10	5–10	5–10	2.5	2.5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	1.25	5–10	5–10	5–10	5–10	0.25	0.25	0.031
17	5–10	5–10	0.15	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2.5	5–10	5–10	0.3	5–10	5–10	5–10	5–10	5–10	5–10	0.25	0.125	0.125
18	1.25	2.5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.6	0.3	0.3	5–10	0.3	1.25	1.25	2.5	5–10	2.5	0.25	0.25	0.125
19	0.6	1.25	1.25	5–10	5–10	5–10	5–10	0.02	0.3	0.6	0.3	0.08	0.6	0.3	0.3	0.6	0.08	0.6	1.25	1.25	2.5	0.6	0.25	0.125	0.06
20	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2.5	0.6	5–10	5–10	0.6	5–10	5–10	2.5	5–10	5–10	0.125	0.125	0.06
21	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	2.5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	0.25	0.125
22	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.3	2.5	5–10	5–10	5–10	5–10	5–10	5–10	5–10	5–10	0.5	0.25	0.25
Chloram**phenicol**	0.005	0.005	0.005	0.005	0.0012	0.005	0.0012																		
Tetracycline	0.005	0.04	0.01	0.005	0.0012	0.005	0.0012																		
Nystatin								0.6	0.04	0.125	0.6	0.04	0.02	0.15	0.08	0.08	0.08	0.3	0.15	0.6	0.6	0.6			
Ketoconazole								0.04	0.04	0.04	0.01	0.04	0.04	0.15	0.02	0.04	0.01	0.04	0.04	0.04	0.04	0.04			
Amphotericin B								0.04	0.02	0.02	0.02	0.01	0.04	0.04	0.04	0.04	0.02	0.04	0.04	0.04	0.04	0.02			
Metconazole																							0.031	0.007	0.007
Tebuconazole																							0.015	0.0017	0.007

§ A, C, D, E, H, I, J, O – isolates from vagina; B, F, L, N – isolates from urine; G, M – isolates from sputum; K – isolate from throat

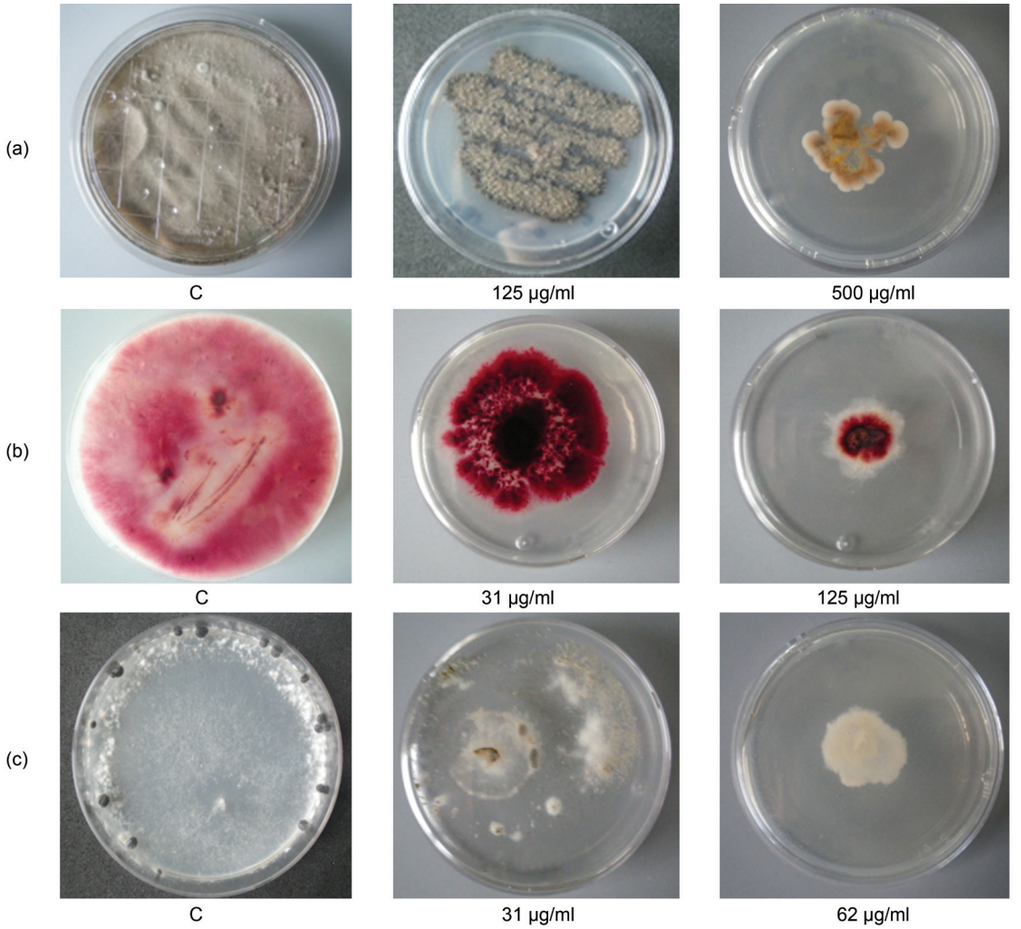


Fig. 1. Influence of presented compounds on spawn development of phytopathogens.
(a) *C. gloeosporioides*, (b) *F. culmorum*, (c) *S. sclerotiorum* were grown in the presence of compound 4, 16 and 22 respectively.

compounds (18 and 19) inhibited the growth of this strain with MIC values in the range of 0.6–1.25 mg/ml making them promising hits for future development. Growth of certain *C. tropicalis* strains was also inhibited by compounds 3, 11, 16, 18, and 20 with MIC's in the range of 1.25–2.5 mg/ml. Although, only compound 19 with three hydroxyl substituted benzene moiety at the diazole ring and two methyl substituents on the aromatic ring was able to effectively inhibit all strains of *Candida* used in this study (MIC = 0.6–2.5 mg/ml). The fact that compound 19 successfully inhibits growth of all tested strains is of great importance because it can lead in the future to design agents with broad antifungal activity. Similar activities were obtained in the case of bis-5-methylbenzimidazoles (Küçükbay *et al.*, 2003a) and electron-rich olefins derived benzimidazoles (Küçükbay *et al.*, 2003b) against *C. albicans* and *C. tropicalis*. When nitro- and halogen-benzimidazoles were substituted with long-chain alkyl acids they gained higher potency up to 37.5 µg/ml of MIC value (Sharma *et al.*, 2009). The spectrum of infections caused by *Candida* is very extensive and includes both superficial and deep infections. Superficial infection in favorable conditions can develop in the skin, the epidermis and mucous membranes. An important clinical problem is the deep infections that may take the form of organ, systemic or disseminated infetions, and at the same time have high, over 70% mortality. *Candida* species are the fourth most common pathogen isolated from the blood of hospitalized patients. Thus, it is justified to design and synthesize new antifungal agents.

Interesting results were obtained in the case of plant pathogens. All tested benzimidazoles showed good inhibitory properties against *C. gloeosporioides*, *F. culmorum* and *S. sclerotiorum*. MIC values were found in the range of 0.031–2 mg/ml (Table II). The inhibition of growth of these species is very important from the agriculture point of view as they are characterized by high non-specificity and polifagism and inflict a lot of damage in crop breeding. *S. sclerotiorum* was found to be the most sensitive strain especially to compounds 16–21 with MICs below 0.125 mg/ml. This fact makes those compounds promising output agents as this pathogen is attacking more than 400 species of plants around

the world, including many important crops (oilseeds, pulses, fodder plants, vegetables and ornamental plants). Additionally, there was an evident effect of the presented benzimidazoles on the macroscopic look of the studied fungi colonies. On control plates, abundant, fluffy spawns developed whereas spawns that grown on plates with presence of benzimidazoles were clearly poorer developed (Fig. 1.) what suggests that the presented compounds can effectively inhibit the expansion of fungi spawns. Only compound 15 didn't show such influence.

Referring the obtained results to the chemical structure of active compounds some common characteristics that determine biological activity should be indicated. Such activity is undoubtedly connected with the permeability of membranes and cell walls of microorganisms. Better anti-microbial properties are mainly demonstrated by benzimidazoles with greater hydrophilicity, that is lower logP (compounds 17–22). Such tendency is also seen regarding the molecular weight, where smaller compounds (MW < 300 kDa) present better activity. Reference of the obtained results to the chemical structure of the tested compounds broadens the knowledge of the practical use of new biologically active substances containing benzimidazole ring and gives rise to possible chemical modification of compounds in order to achieve greater effectiveness in inhibiting the growth of undesirable microorganisms.

In view of the above findings, the presented candidates may be valuable in designing more potent and selective anti-microbial agents serving as promising starting templates.

Literature

Andrzejewska M., L. Yepez-Mulia, A. Tapia, R. Cedillo-Rivera, A.E. Laudy, B.J. Starościak and Z. Kazimierczuk. 2004. Synthesis, and antiprotozoal and antibacterial activities of S-substituted 4,6-dibromo- and 4,6-dichloro-2-mercaptobenzimidazoles. *Eur. J. Pharm. Sci.* 21: 323–329.

Bansal Y. and O. Silakari. 2012**.** The therapeutic journey of benzimidazoles: a review. *Bioorg. Med. Chem.* 20: 6208–6236.

Gaba M., S. Singh and C. Mohan. 2014. Benzimidazole: an emerging scaffold for analgesic and anti-inflammatory agents. *Eur. J. Med. Chem.* 76: 494–505.

Gianoncelli A., G. Cozza, A. Orzeszko, F. Meggio, Z. Kazimierczuk and L. A. Pinna. 2009. Tetraiodobenzimidazoles are potent inhibitors of protein kinase CK2. *Bioorg. Med. Chem.* 17: 7281–7289.

Horton D.A., G.T. Bourne and M. L. Smythe. 2003. The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.* 103: 893–930.

Janeczko M., A. Orzeszko, Z. Kazimierczuk, R. Szyszka and A. Baier. 2012. CK2α and CK2α' subunits differ in their sensitivity to 4,5,6,7-tetrabromo- and 4,5,6,7-tetraiodo-1H-benzimidazole derivatives. *Eur. J. Med. Chem.* 47: 345–350.

Kaplancikli Z.A., G. Turan-Zitouni, G. Revial and K. Guven. 2004**.** Synthesis and study of antibacterial and antifungal activities of novel 2-[[(benzoxazole/benzimidazole-2-yl)sulfanyl] acetylamino] thiazoles. *Arch. Pharm. Res.* 27: 1081–1085.

Karpińka M.M., J. Matysiak and A. Niewiadomy. 2011**.** Synthesis of novel 4-(1H-benzimidazol-2-yl)benzene-1,3-diols and their cytotoxic activity against human cancer cell lines. *Arch. Pharm. Res.* 34: 1639–1647.

Karpińska M.M., J. Matysiak, A. Niewiadomy, J. Wietrzyk and D. Kłopotowska. 2012. Synthesis and biological activity of novel 4- and 6-(1-alkyl/aryl-1H-benzimidazol-2-yl)benzene-1,3-diols *Monatshefte für Chemie* 143: 269–276.

Kazimierczuk Z., M. Andrzejewska, J. Kaustova and V. Klimesova. 2005. Synthesis and antimycobacterial activity of 2-substituted halogenobenzimidazoles. *Eur. J. Med. Chem.* 40: 203–208.

Küçükbay H., R. Durmaz, N. Okyucu and S. Günal. 2003a. Antifungal activity of some bis-5-methylbenzimidazole compounds. *Folia Microbiol* (*Praha*) 48: 679–681.

Küçükbay H., R. Durmaz, E. Orhan and S. Günal. 2003b. Synthesis, antibacterial and antifungal activities of electron-rich olefins derived benzimidazole compounds. *Farmaco* 58: 431–437.

Laudy A.E., R. Moo-Puc, R. Cedillo-Rivera, Z. Kazimierczuk and A. Orzeszko. 2012. Synthesis and antimicrobial activities of new polyhalogenated benzimidazoles. *Journal of Heterocyclic Chemistry* 49: 1059–1063.

Los R., M. Wesołowska-Trojanowska, A.M. Malm, M. Karpińska, J. Matysiak, A. Niewiadomy and U. Głaszcz. 2012. A new approach to the synthesis of 2-aryl-substituted benzimidazoles, quinazolines, and other related compounds and their antibacterial activity. *Heteroatom Chemistry* 23:265–275.

Martin R.J. 1997. Modes of action of anthelmintic drugs. *Vet. J.* 154: 11–34.

Navarrete-Vázquez G., M.e.M. Rojano-Vilchis, L. Yépez-Mulia, V. Meléndez, L. Gerena, A. Hernández-Campos, R. Castillo and F. Hernández-Luis. 2006. Synthesis and antiprotozoal activity of some 2-(trifluoromethyl)-1H-benzimidazole bioisosteres. *Eur. J. Med. Chem.* 41:135–141.

Padmavathi V., P. Thriveni, G. Sudhakar Reddy and D. Deepti. 2008. Synthesis and antimicrobial activity of novel sulfone-linked bis heterocycles. *Eur. J. Med. Chem.* 43: 917–924.

Pagano M.A., M. Andrzejewska, M. Ruzzene, S. Sarno, L. Cesaro, J. Bain, M. Elliott, F. Meggio, Z. Kazimierczuk and others. 2004. Optimization of protein kinase CK2 inhibitors derived from 4,5,6,7-tetrabromobenzimidazole. *J. Med .Chem*. 47: 6239–6247.

Pedini M., G. Alunni Bistocchi, A. Ricci, L. Bastianini and E. Lepri. 1994. New heterocyclic derivatives of benzimidazole with germicidal activity-XII--Synthesis of N1-glycosyl-2-furyl benzimidazoles. *Farmaco* 49: 823–827.

Sharma S., S. Gangal and A. Rauf. 2009. Convenient one-pot synthesis of novel 2-substituted benzimidazoles, tetrahydrobenzimidazoles and imidazoles and evaluation of their in vitro antibacterial and antifungal activities. *Eur. J. Med. Chem.* 44: 1751–1757.

Shingalapur R.V., K.M. Hosamani and R.S. Keri. 2009. Synthesis and evaluation of in vitro anti-microbial and anti-tubercular activity of 2-styryl benzimidazoles. *Eur. J. Med. Chem.* 44: 4244–4248.

Tunçbilek M., T. Kiper and N. Altanlar. 2009. Synthesis and in vitro antimicrobial activity of some novel substituted benzimidazole derivatives having potent activity against MRSA. *Eur. J. Med. Chem.* 44: 1024–1033.

Vinodkumar R., S.D. Vaidya, B.V. Siva Kumar, U.N. Bhise, S.B. Bhirud and U.C. Mashelkar. 2008. Synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel N-substituted-2-(4-phenylethynyl-phenyl)-1H-benzimidazoles and N-substituted 2[4-(4,4-dimethyl-thioc hroman-6-yl-ethynyl)-phenyl)-1H-benzimidazoles. *Eur. J. Med. Chem.* 43: 986–995.

Zacny V.L., E. Gershburg, M.G. Davis, K.K. Biron and J.S. Pagano. 1999. Inhibition of Epstein-Barr virus replication by a benzimidazole L-riboside: novel antiviral mechanism of 5, 6-dichloro-2-(isopropylamino)-1-beta-L-ribofuranosyl-1H-benzimidazole. *J. Virol.* 73: 7271–7277.