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Anti-infective and herbicidal activity of N-substituted 2-aminobenzothiazoles

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ABSTRACT

In this study, a series of N-substituted 2-aminobenzothiazoles was prepared according to a recently developed method. Twelve compounds were tested for their activity related to the inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. Primary in vitro screening of the discussed compounds was also performed against fungal, bacterial and mycobacterial species. The biological activities of some compounds were comparable or higher than the standards phenoxymethylpenicillin or pyrazinamide. The most effective compounds demonstrated insignificant toxicity against the human monocytic leukemia THP-1 cell line. For all compounds, the structure-activity relationships are discussed.

1. Introduction

2-Aminobenzothiazoles substituted on the endocyclic nitrogen have been studied for their potential biological activities since the early 1940s when Institute for Chemotherapy in Germany examined their effects against several infectious diseases. The primary objective was to compare benzothiazoles with thiazoles which were already known and used as drugs, especially sulfathiazole being one of the most efficient drugs for various disorders caused by cocci at that time.[1] The heterocyclic system of benzothiazoles might be substituted in many ways and provide compounds with interesting and even unexpected properties. Among them, the most important is modification of position 2. Diverse biological activities such as antibacterial,[2] antiparasitic,[3,4] antifungal,[5,6] anticancer,[7–10] anti-inflammatory/antiallergic[11–13] and antioxidant[14] were described. Benzothiazoles are also known as potent inhibitors of various enzymes, for example phosphodiesterase[15] and serine hydrolases.[16] They showed activity against some neuronal diseases[17,18] and exhibited significant blood pressure reduction and increase of myocardial perfusion.[19] Moreover, the basic 2-substituted-1,3-benzothiazole scaffold is essential for some herbicides, plant desiccants and defoliant compounds.[20] Isopropyl [(*S*)-1-[(*R*)-1-(6-flourobenzothiazolel-2-yl)ethylcarbamoyl]-2-methylpropyl]-carbamate is a commercially used fungicide which is effective for controlling the oomycete fungal pathogen *Plasmopara viticola*, which causes downy mildew in grapevines.[21] Generally, benzothiazoles posses a unique and versatile scaffold for experimental drug design.

The presence of an amide group is characteristic of a number of herbicidal active compounds[22–30] that were also found to be uncouplers of photosynthetic phosphorylation.[31] Over 50% of commercially available herbicides act by reversibly binding to photosystem II (PS II), a membrane-protein complex in the thylakoid membranes, which catalyses the oxidation of water and the reduction of plastoquinone,[32] and thereby inhibit photosynthesis.[33–35]

Both pharmaceuticals and pesticides are designed to target particular biological functions and, in some cases, these functions overlap in their molecular target sites or they target similar

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processes or molecules. Modern herbicides express low toxicity against mammals and one of the reasons is that mammals lack many of the target sites for herbicide action. At present, approximately 20 mechanisms of action of herbicides are known. It was determined that inhibitors of protoporphyrinogen oxidase, 4-hydroxyphenylpyruvate dioxygenase and glutamine synthetase inhibit these enzymes both in plants and mammals. However, the consequences of inhibition of the overlapping target site can be completely different. Therefore, a compound that has lethal action on plants may be beneficial for mammals.[36] Such chemicals are characterized by low toxicity on mammals as a result of quick metabolism and/or elimination of herbicide from the mammalian system. Taking into consideration that herbicides may also have molecular sites of action in mammals, until recently most pharmaceutical companies had pesticide divisions, sometimes with a different name. All compounds generated by either division of the company were evaluated for both pesticide and pharmaceutical uses. In the past, some leading pesticides have become pharmaceuticals and vice versa. However, little information of this type was published and must usually be deduced from patent literature. One of the exceptions is fluconazole, a fungicide product discovered by the pharmaceutical sector that is now used both as a pharmaceutical and also patented as a chemical with applications in crop production.[36–38]

As mentioned above, position 2 of benzothiazoles is the most suitable for affecting physico-chemical properties of these compounds.[16] Following a recently developed method[39] for the synthesis of 2-aminobenzothiazoles, the amino moiety was modified to obtain biologically effective derivatives and to further explore the scope of the synthetic approach. A series of twelve compounds was tested for inhibitory effects on photosynthetic electron transport (PET) in spinach chloroplasts (*Spinacia oleracea* L.) and also evaluated against fungal, bacterial and mycobacterial strains. As many low-molecular-weight drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity, the experimental log*k* values were determined by high performance liquid chromatography. The structure–activity relationships between the chemical structure, physical properties and in vitro biological activities of all the evaluated compounds are discussed.

2. Results and discussion

2.1. Chemistry

There are many methods for the preparation of 2-aminobenzothiazoles. To avoid working with toxic and inconvenient (2-aminothiophenol, diazonium salts) or expensive (transition metal complexes) starting materials, the procedure reported previously was chosen.[39] Six derivatives **3a–c**, **3e**, **3j** and **3l** were, therefore, available in yields up to 93%, and six new compounds were synthesized now to generate a well-organized set (small library)—Scheme 1. Unsubstituted 2-aminobenzothiazole **3a** (R = H) was obtained by basic hydrolysis of the benzoyl derivative **3j**.

Attempts to extend the amines series with *tert*-butyl and cyclohexyl derivatives were unsuccessful. This might be caused by steric hindrance on the isothiocyanate carbon that prevented 2,2′-diaminodiphenyl disulfide from nucleophilic addition. Similarly, in the set of amides, the yield of *tert*-butyl **3h**, *iso*-butyl **3g** and butyl derivative **3f** was poor, moderate and very good, respectively, and 2-phenylacetylaminobenzothiazole **3k** was obtained in a higher yield than 2-cyclohexylcarbonylaminobenzothiazole **3i**. The presence of the carbonyl group further supported the reaction conversion by its electron withdrawing effects. The best results exceeding 70% were, therefore, achieved in the amide series when a substituent providing enough space for the interaction with 2,2′-diaminodiphenyl disulfide was introduced (**3f** and **3k**).

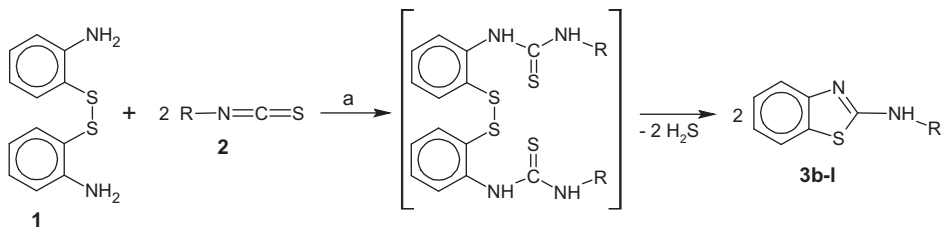
Although some difficulties during the preparation (oligomerization of isothiocyanates) might occur, this recently developed synthetic approach proved useful, simple and convenient.

2.2. Physico-chemical properties

Lipophilicity is a property that has a major effect on absorption, distribution, metabolism, excretion and toxicity properties as well as pharmacological activity, because drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity. Lipophilicity has been studied and applied as an important drug property for decades.

Hydrophobicities (log*P*/C log*P*) of compounds **3a–l** were calculated using two commercially available programs (ChemOffice Ultra 10.0 and ACD/Log*P* ver. 1.0) and measured by means of RP-HPLC determination of capacity factors *k* with subsequent calculation of log*k*. The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped non-polar C_{18} stationary RP column. The results are shown in Table 1 and illustrated in Figure 1.

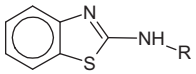
The results obtained with all the compounds show that the experimentally-determined lipophilicities (log*k*) of the discussed compounds are in accordance with the calculated values of compounds **3a–l** as shown in Figure 1. The minimum match of experimental (log*k*) versus calculated (log*P*) values was found for ACD/Log*P*, see Figure 1**B**. As expected, unsubstituted 1,3-benzothiazol-2-amine **3a** showed the lowest lipophilicity (log*k*), while compounds **3i** and **3c** exhibited the highest log*k* values. Generally, the introduction of a carbonyl moiety (compounds **3c–3k**) caused a decrease in lipophilicity in comparison with amino derivatives, for example **3e** < **3b**, **3k** < **3d**, **3j** < **3c**. Benzyl-substituted compounds **3d** and **3k** possessed lower lipophilicity than phenyl derivatives **3c** and **3j**. Within the series of butyl-substituted compounds **3f–h**, the determined log*k* values increased as follows: *iso*-Bu (**3g**) < Bu (**3g**) < *tert*-Bu (**3h**). This experimental data corresponds



R = Me (**3b**), Ph (**3c**), Bn (**3d**),
COMe (**3e**), COBu (**3f**), CO*i*-Bu (**3g**), CO*t*-Bu (**3h**), CO*c*-Hx (**3i**), COPh (**3j**), COBn (**3k**),
COOMe (**3l**)

Scheme 1. Synthesis of N-substituted 2-aminobenzothiazoles **3b–l**: (a) various conditions, see Section 4.

Table 1
Structure of discussed compounds and comparison of calculated lipophilicities (log*P*/*C*log*P*) with determined log*k* values and bulk parameter (MR, reflecting bulkiness)



	R	Log*k*	Log*P*/*C*log*P* ChemOffice	Log*P* ACD/Log*P*	MR (cm^3/mol) Chemoffice
3a	H	0.3072	2.43/1.8230	1.89 ± 0.57	1.60
3b	Me	0.3819	2.73/2.6266	2.23 ± 0.57	6.80
3c	Ph	0.7301	4.70/4.6056	3.98 ± 0.57	25.28
3d	Bn	0.6012	4.46/4.0746	4.00 ± 0.57	31.10
3e	CO-Me	0.3661	2.14/1.9914	1.84 ± 0.60	11.58
3f	CO-Bu	0.5780	3.63/3.5784	3.44 ± 0.60	25.53
3g	CO-*i*Bu	0.5598	3.54/3.4484	3.25 ± 0.60	25.70
3h	CO-*t*Bu	0.5891	4.07/3.2284	3.07 ± 0.61	25.79
3i	CO-*c*Hx	0.6880	4.11/4.0224	3.90 ± 0.60	32.77
3j	CO-Ph	0.6643	4.04/3.4804	3.30 ± 0.60	32.43
3k	CO-Bn	0.5413	3.98/3.5294	3.62 ± 0.61	36.02
3l	CO-OMe	0.4251	2.72/2.2765	2.29 ± 0.61	12.89

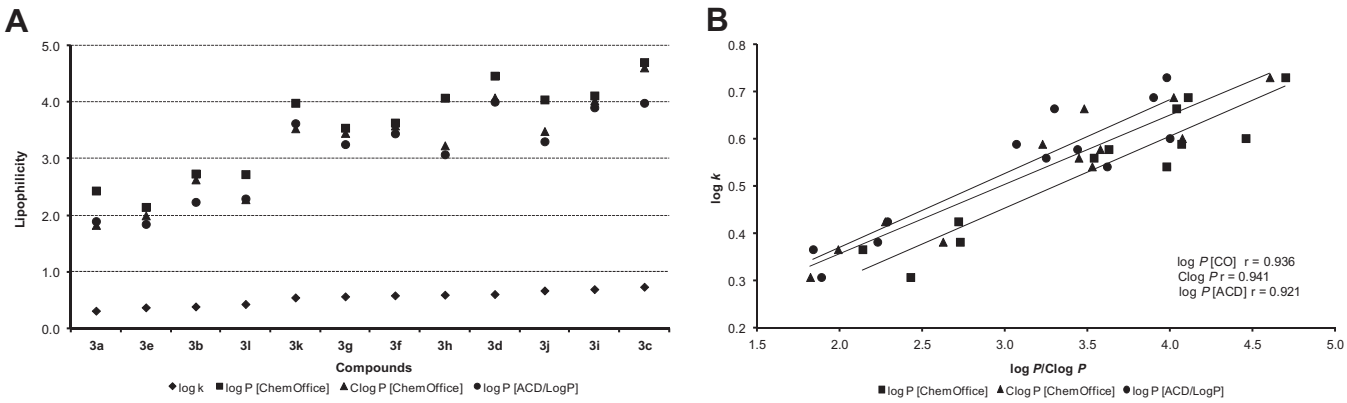


Figure 1. Comparison of log*P* data calculated using two programs with experimentally found log*k* values. Fig. 1A: compounds are arranged in ascending manner according to experimental log*k* values; Fig. 1B: match of calculated data with experimentally found log*k* values.

to only log*P* calculated by ChemOffice. Generally, it can be assumed that log*k* values specify lipophilicity within individual series of the studied compounds more precisely than calculated log*P*/*C*log*P* data.

Experience has shown that a parameter representing the volume of substituents on each compound relative to other members of the same series may often be correlated with the biological data. Of the various bulk parameters proposed, molar refraction reflecting bulkiness (MR) was chosen. These values are not dependent on the position of the substituent and can be calculated by various chemical software, similarly as log*P*. In Table 1 there are the MR values calculated by ChemOffice Ultra 10.0, and the correlation between experimental log*k* and calculated MR is shown in Figure 2 and the following equation:

$$\log k = 0.304\ (\pm 0.048)\ + 0.010(\pm 0.002)\mathrm{MR}$$

$$r = 0.861, s = 0.073, F = 28.55, n = 12$$

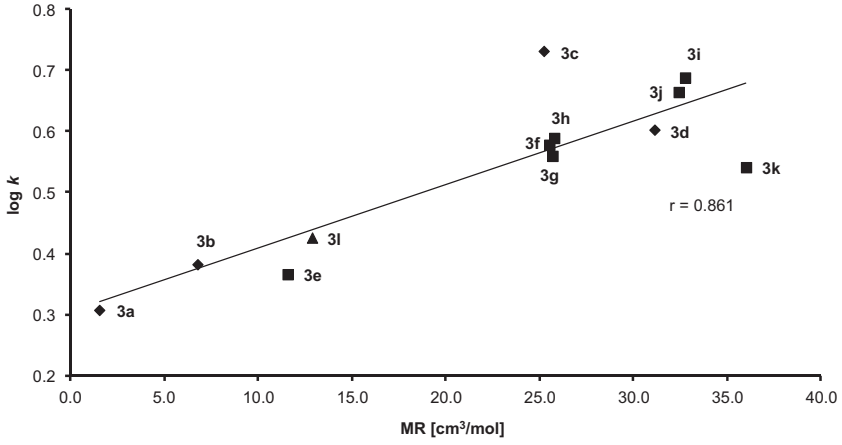


Figure 2. Match of calculated bulk parameters expressed as MR (reflecting bulkiness) data with experimentally found log*k* values. (rhombs = amines, squares = amides, triangle = carbamate).

2.3. Biological activities

The compounds under investigation could be divided into three groups based on their chemical structure: Group 1 includes amines **3a**–**d**; Group 2 containes carboxamides **3e**–**k**; and Group 3 includes carbamate **3l**. Compounds within Group 1 and Group 2 can also be divided according to whether they contain an aromatic or a non-aromatic substituent. The compounds showed a wide range of biological activities, and some interesting structure-activity relationships were observed. All the results are summarized in Table 2.

2.3.1. Inhibition of photosynthetic electron transport (PET) in spinach chloroplasts

The activity of all the evaluated derivatives related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts was moderate or rather low relative to the standard, see Table 2. Only 2-phenylacetylaminobenzothiazole **3k** expressed high PET-inhibiting activity (IC_{50} = 1.11 μmol/L) which is higher than DCMU. Despite the relatively low inhibitory activity of the rest of the studied compounds, the structure-activity relationships can be discussed. Generally, Group 1 (amines **3a**–**d**) showed lower PET-inhibiting activity than the carboxamides or the carbamate, that is –NH-Me (**3b**) < –NHCO-Me (**3e**) < –NHCO-OMe (**3l**). The carbamate moiety (Group 3) seems to be the most advantageous as was described recently.[27,29]

It can be stated that the dependence of PET-inhibiting activity on the lipophilicity was linear up to log*k* = 0.54 and with the further increase of log*k* it gradually decreased. The most active compound phenylacetamide **3k** has a lipophilicity optimum at log *k* = 0.54. Within the series of 2-substituted benzothiazoles the length and branching of the chain seem to be also important. It is evident that PET inhibition increases with the chain length (–COC_4H_9, **3f**) or mono-β-branched (–$COCH_2C_6H_5$, **3k**) substituent, while in the case of short chain (compounds **3a**,**b**,**e**) or α-branched substituent as *tert*-butyl **3h**, cyclohexyl **3i** or phenyl **3c**,**j** PET inhibition dramatically decreases. It is evident that the bulkiness of the *N*-substituents influences PET-inhibiting activity. Excellent PET inhibition by benzyl substituted amides has also been recently described by Otevrel et al. and Gonec et al.[27,30]

Several series of alkyl substituted benzothiazole derivatives were also recently investigated for their photosynthesis-inhibiting activity.[40–42] PET inhibition in spinach chloroplasts was also observed for 2-(6-acetamidobenzothiazolethio)acetic acid esters.[43] The dependence of PET-inhibiting activity on the lipophilicity of the derivatives with R = *n*-alkyl and allyl showed a quasiparabolic course, the most active compound was the hexyl derivative (IC_{50} = 47 μmol/L). The effect of 2-(alkoxycarbonylmethylthio)-6-

Table 2
IC_{50} values related to PET inhibition in spinach chloroplasts of N-substituted 2-aminobenzothiazoles in comparison with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) standard; in vitro antifungal activity (IC_{80}/IC_{50}) of compounds compared to fluconazole (FLU) standard; in vitro antibacterial activity (IC_{80}) of compounds compared to phenoxymethylpenicillin (PMP) standard; in vitro antimycobacterial activity (IC_{90}) of compounds in comparison with isoniazid (INH) and pyrazinamide (PZA) standards; and in vitro cytotoxicity assay (LD_{50}) of choice compounds

		[μmol/L]							
	PET IC_{50}	MIC/IC_{80}[a]/IC_{50}[b]				MIC/IC_{80}		MIC/IC_{90}	LD_{50}
		CA[a]	CK[a]	TA[a]	TM[b]	MRSA	SE	MK	
		24 h 48 h	24 h 48 h	24 h 48 h	72 h 120 h	24 h 48 h	24 h 48 h	5 days	
3a	1722	>500 >500	>500 >500	>500 >500	>500 >500	>500 >500	>500 >500	>1997	–
3b	364.4	125 250	500 >500	125 >500	62.50 62.50	500 500	125 125	>1827	–
3c	57.7	ND	ND	ND	ND	ND	ND	ND	–
3d	ND	>125 >125	>125 >125	125 >125	>125 >125	>125 >125	>125 >125	ND	–
3e	86.4	62.50 125	250 >500	250 >500	250 250	250 500	250 >500	1300	>30
3f	21.0	31.25 31.25	125 >125	62.50 >125	31.25 62.50	31.25 125	31.25 62.50	1087	>30
3g	34.1	125 125	>125 >125	125 >125	62.50 62.50	125 125	>125 >125	480	>30
3h	74.6	62.50 250	500 >500	>500 >500	62.50 250	250 500	125 500	1040	ND
3i	113.2	>125 >125	>125 >125	125 >125	>125 >125	>125 >125	>125 >125	960	>30
3j	77.3	125 125	500 >500	250 >500	62.25 125	125 500	>500 >500	533	>30
3k	1.11	3.90 7.81	62.50 125	31.25 62.25	15.62 125	31.25 62.25	125 500	256	>30
3l	24.2	125 250	>250 >250	250 500	125 125	250 250	125 500	533	27.5
DCMU	1.9	–	–	–	–	–	–	–	–
FLU	–	0.06 0.12	3.91 15.62	0.24 0.48	1.95 3.91	–	–	–	–
PMP	–	–	–	–	–	250 500	62.50 250	–	–
INH	–	–	–	–	–	–	–	<72.9	–
PZA	–	–	–	–	–	–	–	>812	–

ND = not determined due to precipitation during the experiment or interaction with 2,6-dichlorophenol-indophenol (DCPIP).
The MIC determination was performed according to the CLSI reference protocol:
[a] M27-A2 for yeasts (IC_{80} value).
[b] M38-A for moulds (IC_{50} value); CA = *Candida albicans* ATCC 44859, CK = *C. krusei* E28, TA = *Trichosporon asahii* 1188, TM = *Trichophyton mentagrophytes* 445, MRSA = methicillin-resistant *Staphylococcus aureus* H 5996/08, SE = *S. epidermidis* H 6966/08, MK = *Mycobacterium kansasii* CIT11/06.

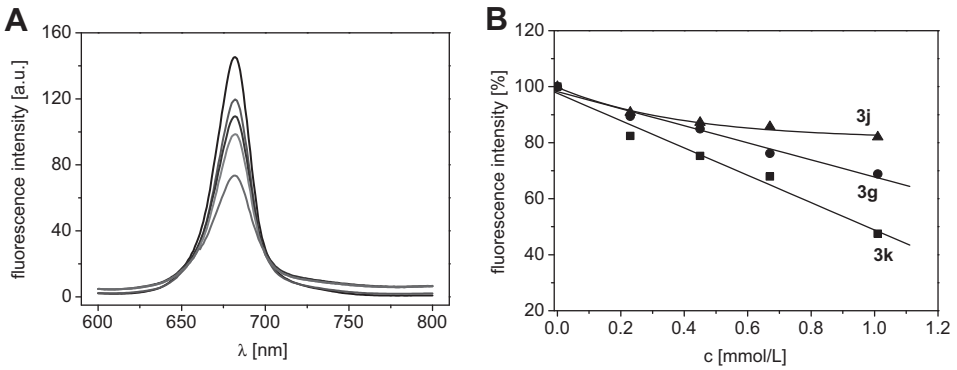


Figure 3. Emission fluorescence spectra of chlorophyll *a* in suspension of spinach chloroplasts without and with 0.23, 0.45, 0.67 and 1.00 mmol/L of *N*-1,3-benzothiazol-2-yl-2-phenylacetamide **3k** (curves from top to bottom) (A), and dependence of intensity of chlorophyll *a* fluorescence emission band at 686 nm in suspension of spinach chloroplasts on concentration of studied compounds **3k** (square), **3g** (ring) and **3j** (triangle) (B) ($_{ex}$ = 436 nm).

aminobenzothiazoles on photosynthetic apparatus was similar to 2-alkylthio-6-*R*-benzothiazoles, where R = 6-formamido-, 6-acetamido- and 6-benzoylamino–, and as a probable site of their action the oxygen evolving complex was suggested.[44]

Because the studied compounds inhibited Hill reaction, they can be considered to be PS II herbicides. Upon addition of diphenylcarbazide (DPC, an artificial electron donor with the known site of action in the intermediate Z/D on the donor side of PS II[45]) to chloroplasts, activity of which was inhibited by **3k**, PET was practically completely restored. Thus, it can be assumed this benzothiazole derivatives did not damage PET between photosynthetic centres PS II (P 680) and PS I (P 700), and their site of action is situated at the donor side of PS II. The site of action situated at the donor side of PS II was found also for 2-alkylthio-6-*R*-benzothiazoles.[41]

The effects of studied compounds on the photosynthetic centres of chloroplasts were investigated by studying chlorophyll *a* fluorescence. Figure 3A shows how the chlorophyll *a* fluorescence emission band at 686 nm is influenced in the presence of *N*-1,3-benzothiazol-2-yl-2-phenylacetamide. The decreased intensity of this emission band belonging to the chlorophyll-protein complexes mainly in photosystem (PS) II[46] suggested PS II as the site of action of the studied inhibitor. As shown in Figure 3B, the intensity of the chlorophyll *a* fluorescence emission band at 686 nm in the suspension of spinach chloroplasts decreased with increasing concentration of the studied compounds and the rate of fluorescence decline correlated with the effectiveness of PET inhibition. The greatest decrease was observed with the most effective PET inhibitor **3k** (R = CO-Bn, IC_{50} = 1.1 μmol/L), in the presence of **3g** (R = CO-*i*Bu, IC_{50} = 34.1 μmol/L) the fluorescence intensity was affected to a lesser extent, and even less effect was observed with **3j** (R = CO-Ph, IC_{50} = 77.3 μmol/L).

2.3.2. In vitro antifungal susceptibility testing

The evaluation of in vitro antifungal activity of the synthesized compounds was performed against *Candida albicans* ATCC 44859 (CA), *Candida krusei* E28 (CK), *Trichosporon asahii* 1188 (TA) and *Trichophyton mentagrophytes* 445 (TM), and the results are shown in Table 2. All the compounds expressed only moderate antifungal activity, except *N*-1,3-benzothiazol-2-yl-2-phenylacetamide (**3k**). Generally, it can be concluded that Group 1 did not possess any antifungal activity in comparison with the amides (Group 2) or the carbamate (Group 3). It can be stated that the dependence of in vitro antifungal activity on the lipophilicity seems to be important; nevertheless, as discussed above, substituent bulkiness also plays a significant role. Although in the case of anti-CA activity it is evident that amide substituted by benzyl moiety (compound **3k** with a lipophilicity optimum at log*k* = 0.54) is the most advantageous, in the case of anti-TM activity 2-pentanoylaminobenzothiazole (**3f**) with lipophilicity log*k* = 0.58 was the most efficient. In this case a longer (C_4) chain (**3f**) that can be monobranched in β-position (**3g**) or unsaturated (**3k**), the radical analogue of **3g**, increases anti-TM activity. A short chain or α-branched (*tert*-butyl, cyclohexyl, phenyl) chain caused a decrease in activity.

2.3.3. In vitro antibacterial susceptibility testing

All compounds were tested for their in vitro antibacterial activity against two Gram positive bacterial strains methicillin-resistant *Staphylococcus aureus* H 5996/08 (MRSA) and *Staphylococcus epidermidis* H6966/08 (SE). The results are shown in Table 2. Generally, all the compounds expressed only moderate antibacterial activity, apart from 2-phenylacetylaminobenzothiazole (**3k**) and 2-pentanoylaminobenzothiazole (**3f**), where the antibacterial activity is comparable with or higher than the standard phenoxymethylpenicillin against methicillin-resistant *S. aureus*. Similarly as mentioned above, it can be concluded that the amines (Group 1) did not possess any antibacterial activity in comparison with the amides (Group 2) or the carbamate (Group 3).

The dependences of the antibacterial activity of compounds **3b**,**e**–**h**,**j**–**l** against methicillin-resistant *S. aureus* on lipophilicity (log*k*) was performed (see Fig. 4). Based on the results obtained it is possible to declare that lipophilicity (optimum at log*k* = 0.54) is the most important parameter. Similarly, based on Table 2, it can be assumed that antibacterial activity against *S. aureus* is also affected by bulk parameters expressed as MR of R substituents, when double-branching on the α-position of a substituent (i.e. *tert*-butyl (MR = 25.79 cm^3/mol) **3h** and phenyl (MR = 32.43 cm^3/mol) **3j**) caused an expressive decrease in activity. The character of the dependence of anti-MRSA activity on log*k* is the same as the dependence of PET inhibition on lipophilicity, and phenylacetamide **3k** is the most effective compound with log*k* = 0.54.

2.3.4. In vitro antimycobacterial evaluation

Although all the compounds were evaluated for their in vitro antimycobacterial activity against *Mycobacterium kansasii* CIT11/06 (MK), most compounds did not show any activity. Only 2-phenylacetylaminobenzothiazole (**3k**) expressed high activity against *M. kansasii*. Though the antimycobacterial activity of **3k** is less than that of isoniazid, it is threefold higher than the activity of pyrazinamide. Similar trends, as mentioned above, that is lipophilicity (optimum at log*k* = 0.54) as well as the bulkiness of the N-substituents within the amide series, influence antimycobacterial activity.

2.3.5. In vitro cytotoxicity assay

Compounds **3e**–**l** were tested for their in vitro cytotoxicity using a human monocytic leukemia THP-1 cell line. The cytotoxicity was

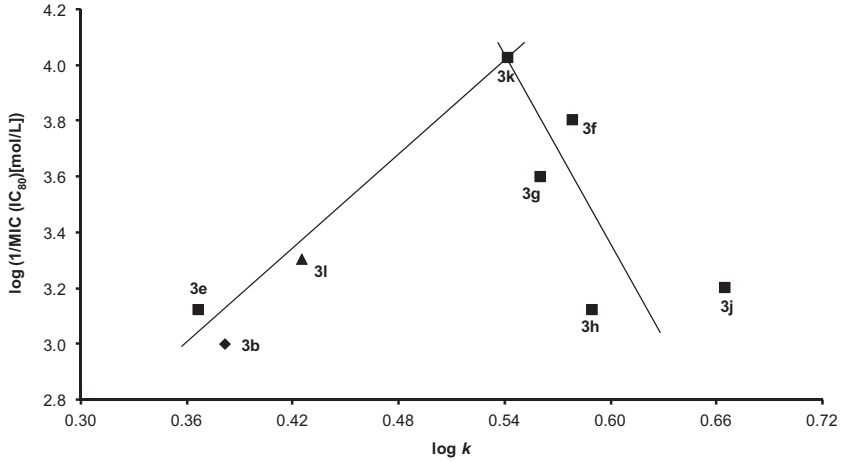


Figure 4. Dependence of in vitro antibacterial activity against methicillin-resistant *S. aureus* (log 1/MIC [mol/L]) on lipophilicity (log*k*) of studied compounds **3a–l**. (rhombs = amines, squares = amides, triangle = carbamate).

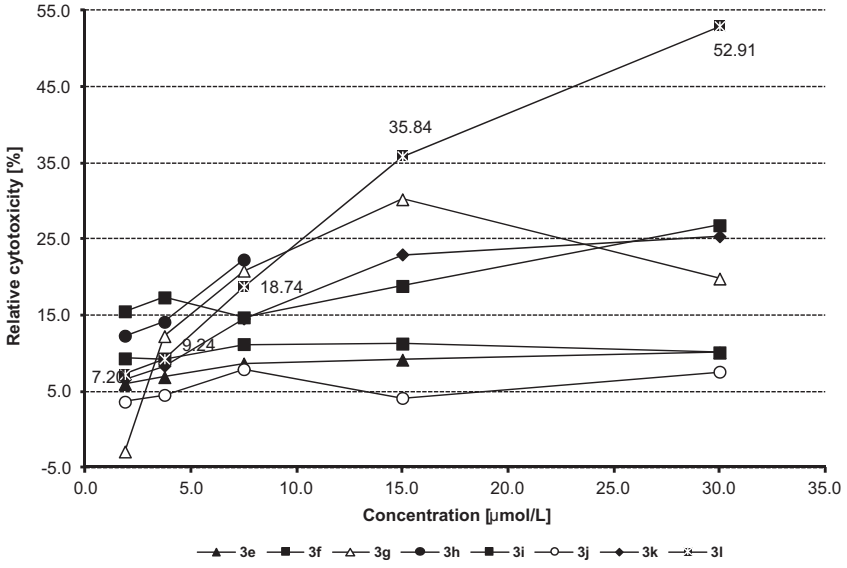


Figure 5. Relative cytotoxicity of compounds **3e–3l** in leukemia THP-1 cell line.

evaluated as the LD_{50} value (LD_{50}—lethal dose to 50% of the cell population). The results are presented in Table 2. Compound **3h** was not soluble in concentrations 15 and 30 μmol/L, the highest concentration prepared in DMSO for the toxicity assay was 7.5 μmol/L. The LD_{50} value for all tested compounds **3e–g** and **3i–k** was determined to be >30 μmol/L. A significant change in viability was found only for compound **3l** with the value of LD_{50} = 27.5 μmol/L for the cell line used in the assay, see Figure 5. It could be derived from the data that none of the compounds tested were cytotoxic in the range of concentrations used in these cytotoxicity assays (e.g. LD_{50} of oxaliplatin 1.7 ± 6.4 μmol/L and camptothecin 0.16 ± 0.07 μmol/L assessed in this line formerly showed much lower values). The value of LD_{50} for all tested compounds was more than 25 μmol/L.

3. Conclusions

Six new N-substituted 2-aminobenzothiazoles were successfully prepared according to the recently developed procedure, which proved to be useful, simple and convenient. In total, twelve compounds were tested for their ability to inhibit photosynthetic electron transport (PET) in spinach chloroplasts (*Spinacia oleracea* L.) and for their in vitro antifungal, antibacterial and antimycobacterial activity. 2-Phenylacetylaminobenzothiazole (**3k**) showed excellent PET inhibition, higher than the standard DCMU. This compound also expressed relatively high antimycobacterial activity against *M. kansasii*. Two compounds, namely 2-phenylacetylaminobenzothiazole (**3k**) and 2-pentanoylaminobenzothiazole (**3f**), expressed high activity against *C. albicans* and methicillin-resistant *S. aureus*. Both exhibited activity comparable with or higher than the standard phenoxymethylpenicillin. Generally, amines **3a–d** showed lower biological activity than carboxamides **3e–3k** or carbamate **3l** indicating that amide/carbamate groups are essential for biological effect. Furthermore, it could be concluded that lipophilicity is fundamental for the biological activities of all compounds in all assays. It can be stated that the dependence of activity on the lipophilicity was parabolic or bilinear. Also, the substitution of an amide moiety by a bulky group or a long alkyl chain is important for high herbicidal or anti-infective activity. The most effective biological compounds **3e–g** and **3i–l** were

tested for their in vitro cytotoxicity against the human monocytic leukemia THP-1 cells. None of the compounds with LD_{50} >25 μmol/L were cytotoxic in the range of concentrations used in these cytotoxicity assays.

4. Experimental

4.1. Chemistry

All reagents and solvents used are commercially available. Reaction conversions were monitored by TLC on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany) in diethyl ether and evaluated under UV (254 nm). Infrared (IR) spectra were recorded on a Smart MIRacle™ ATR ZnSe for Nicolet™ Impact 6700 FT–IR spectrometer (Thermo Scientific, USA). The spectra were obtained by accumulation of 256 scans with 2 cm^{-1} resolution in the region of 4000–600 cm^{-1}. All ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz FT-NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C, Bruker Comp., Karlsruhe, Germany). Chemicals shifts are reported in ppm (δ) using internal $Si(CH_3)_4$ as the reference with diffuse, easily exchangeable signals being omitted. Mass spectra were measured using a LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron Corporation, USA) with direct injection into an APCI source (400 °C) in the positive mode.

Compounds **3a–c**, **3e**, **3j** and **3l** were described and published recently by Fajkusova.[39]

4.2.1. 2-Benzylaminobenzothiazole (3d)

A suspension of 2,2′-diaminodiphenyl disulfide (**1**) (1.5 g, 6.1 mmol) in toluene (30 mL) was treated with benzyl isothiocyanate (1.8 g, 12.1 mmol) and stirred at room temperature. A clear solution was quickly formed; colourless crystals precipitated within 48 h. After filtration the solution was partially evaporated to provide another solid. Both crops were washed with toluene and dried well in the air to give pure **3d** (1.6 g, 55%). IR (cm^{-1}): 3060 (ν, Ar-H), 3030 (ν, Ar-H), 2897 (ν, CH), 2853 (ν_s, CH_2), 1614 (ν, C–N), 1572 (δ, NH), 1453, 1269, 1256, 1235, 753 (γ, Ar), 742 (γ, Ar), 698 (γ, Ar); ^1H NMR ($CDCl_3$), δ: 7.56 (ddd, 1H, J = 0.6 Hz, J = 1.3 Hz, J = 7.9 Hz, Ar), 7.47 (ddd, 1H, J = 0.6 Hz, J = 1.3 Hz, J = 8.1 Hz, Ar), 7.44–7.7.18(m, 6H, Ar), 6.15 (br s, 1H, NH), 4.63 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$), δ: 167.42. 152.31, 137.46, 130.49, 128.91, 128.81, 127.85, 127.66, 127.51, 125.96, 121.61, 120.81, 118.96, 49.38. HR-MS: for $C_{14}H_{13}N_2S$ $[M+H]^+$ calcd 241.07212 m/z, found 241.0791 m/z.

4.2.2. General procedure for synthesis of 2-aminobenzothiazole derivatives 3f–i and 3k

The corresponding acyl chloride was added dropwise to a solution of potassium thiocyanate in dry acetone (20 mL for **3f–h**, 15 mL for **3i** and **3k**) under stirring at room temperature. Potassium chloride precipitated immediately and approx. after 10 min was filtered off and washed with dry acetone. The solution containing the corresponding acyl isothiocyanate was evaporated to dryness. This residue was then dissolved in chloroform (15 mL for **3–h**, 10 mL for **3i** and **3k**), a solid rest was removed and a clear solution added dropwise to a solution of 2,2′-diaminodiphenyl disulfide (**1**) in chloroform (20 mL for **3f–h**, 15 mL for **3i** and **3k**) under stirring at room temperature. Within 2 days the yellow reaction mixture turned pale; hydrogen sulfide evolved. Precipitated crystals were filtered off, and a second crop was obtained by partial evaporation of the solution (ethanol was added as needed). Both portions were washed with chloroform (or ethanol) and dried in the air to provide pure **3f–i** and **3k**. The studied compounds are presented in Table 1.

4.2.3. 2-Pentanoylaminobenzothiazole (3f)

Valeroyl chloride (1.6 g, 13.4 mmol), potassium thiocyanate (1.4 g, 14.8 mmol), **1** (1.0 g, 4.0 mmol). Yield: 1.4 g (74%). IR (cm^{-1}): 3054 (ν, Ar-H), 2951, 2929 (ν_{as}, CH_2), 2867 (ν_s, CH_3), 1695 (ν, C=O), 1600 (ν, C–N), 1548 (δ, NH), 1446, 1379 (δ_s, CH_3), 1272, 1244, 758 (γ, Ar), 724 (γ, CH_2); ^1H NMR ($CDCl_3$), δ: 11.48 (br s, 1H, NH), 7.90–7.80 (m, 1H, Ar), 7.80–7.72 (m, 1H, Ar), 7.52–7.40 (m, 1H, Ar), 7.40–7.28 (m, 1H, Ar), 2.47 (t, 2H, J = 6.7 Hz, $NHCOCH_2CH_2CH_2CH_3$), 1.69 (pent, 2H, J = 7.6 Hz, $NHCOCH_2CH_2CH_2CH_3$), 1.31 (sext, 2H, J = 7.4 Hz, $NHCOCH_2CH_2CH_2CH_3$), 0.86 (t, 3H, J = 7.4 Hz, $NHCOCH_2CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$), δ: 171.87, 159.57, 147.84, 131.99, 126.29, 123.99, 121.61, 120.39, 36.26, 27.01, 22.18, 13.60; HR-MS: for $C_{12}H_{15}N_2OS$ $[M+H]^+$ calcd 235.0827 m/z, found 235.0896 m/z.

4.2.4. 2-(3-Methyl)butanoylaminobenzothiazole (3g)

Isovaleryl chloride (1.6 g, 13.4 mmol), potassium thiocyanate (1.4 g, 14.8 mmol), **1** (1.0 g, 4.0 mmol). Yield: 0.9 g (47%). IR (cm^{-1}): 3055 (ν, Ar-H), 2962 (ν_{as}, CH_3), 2929 (ν_{as}, CH_2), 2868 (ν_s, CH_3), 1697 (ν, C=O), 1599 (ν, C–N), 1538 (δ, NH), 1440, 1268, 1248, 752 (γ, Ar), 728 (γ, CH_2); ^1H NMR ($CDCl_3$), δ: 10.67 (br s, 1H, NH), 7.89–7.82 (m, 1H, Ar), 7.82–7.74 (m, 1H, Ar), 7.52–7.40 (m, 1H, Ar), 7.40–7.28 (m, 1H, Ar), 2.34 (d, 2H, J = 6.7 Hz, $NHCOCH_2CH(CH_3)_2$), 2.16 (non, 1H, J = 6.7 Hz, $NHCOCH_2CH(CH_3)_2$), 0.92 (d, 6H, J = 6.7 Hz, $NHCOCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$), δ: 171.06, 159.11, 147.89, 132.02, 126.26, 124.01, 121.57, 120.58, 45.55, 25.96, 22.26; HR-MS: for $C_{12}H_{15}N_2OS$ $[M+H]^+$ calcd 235.0827 m/z, found 235.0896 m/z.

4.2.5. 2-(2,2-Dimethyl)propanoylaminobenzothiazole (3h)

Trimethylacetyl chloride (1.6 g, 13.4 mmol), potassium thiocyanate (1.4 g, 14.8 mmol), **1** (1.0 g, 4.0 mmol). Yield: 0.2 g (9%). IR (cm^{-1}): 3061 (ν, Ar-H), 2959 (ν_{as}, CH_3), 2868 (ν_s, CH_3), 1686 (ν, C=O), 1601 (ν, C–N), 1537 (δ, NH), 1446, 1298, 1278, 762 (γ, Ar); ^1H NMR ($CDCl_3$), δ: 8.89 (br s, 1H, NH), 7.85–7.79 (m, 1H, Ar), 7.79–7.73 (m, 1H, Ar), 7.50–7.38 (m, 1H, Ar), 7.38–7.27 (m, 1H, Ar), 1.37(s, 9H, $NHCOC(CH_3)_3$); ^{13}C NMR ($CDCl_3$), δ: 176.46, 159.91, 147.96, 132.01, 126.24, 123.93, 121.43, 120.88, 39.35, 27.22; HR-MS: for $C_{12}H_{15}N_2OS$ $[M+H]^+$ calcd 235.0827 m/z, found 235.0896 m/z.

4.2.6. 2-Cyclohexylcarbonylaminobenzothiazole (3i)

Cyclohexanecarbonyl chloride (1.6 g, 10.8 mmol), potassium thiocyanate (1.2 g, 11.8 mmol), **1** (0.8 g, 3.2 mmol). Yield: 0.9 g (53%). IR (cm^{-1}): 3056 (ν, Ar-H), 2927 (ν_{as}, CH_2), 2850 (ν_s, CH_2), 1693 (ν, C=O), 1599 (ν, C–N), 1543 (δ, NH), 1443, 1272, 1262, 1243, 760 (γ, Ar), 733 (γ, CH_2); ^1H NMR ($CDCl_3$), δ: 11.12 (br s, 1H, NH), 7.93–7.66 (m, 1H, Ar), 7.82–7.74 (m, 1H, Ar), 7.54–7.40 (m, 1H, Ar), 7.40–7.28 (m, 1H, Ar), 2.44–2.20 (m, 1H, CH of cyclohexyl), 2.00–0.83 (m, 10H, cyclohexyl); ^{13}C NMR ($CDCl_3$), δ: 174.90, 159.81, 147.84, 132.01, 126.26, 123.99, 121.59, 120.42, 44.96, 29.18, 25.38, 25.13; HR-MS: for $C_{14}H_{17}N_2OS$ $[M+H]^+$ calcd 261.0983 m/z, found 261.1054 m/z.

4.2.7. 2-Phenylacetylaminobenzothiazole (3k)

Phenylacetyl chloride (1.7 g, 10.8 mmol), potassium thiocyanate (1.2 g, 11.8 mmol), **1** (0.8 g, 3.2 mmol). Yield: 1.2 g (71%). IR (cm^{-1}): 3063 (ν, Ar-H), 3038 (ν, Ar-H), 1697 (ν, C=O), 1597 (ν, C–N), 1544 (δ, NH), 1453, 1263, 1239, 757 (γ, Ar), 693 (γ, Ar); ^1H NMR ($CDCl_3$), δ: 9.96 (br s, 1H, NH), 7.87–7.77 (m, 1H, Ar), 7.77–7.68 (m, 1H, Ar), 7.47–7.38 (m, 1H, Ar), 7.38–7.27 (m, 4H, Ar), 7.27–7.16 (m, 2H, Ar), 3.87 (s, 2H, $NHCOCH_2Ph$); ^{13}C NMR ($CDCl_3$), δ: 169.38, 158.49, 148.01, 132.69, 132.12, 129.40, 129.28, 128.00, 126.35, 124.10, 121.52, 120.69, 43.49; HR-MS: for $C_{15}H_{13}N_2OS$ $[M+H]^+$ calcd 269.3336 m/z, found 269.0741 m/z.

4.2. Lipophilicity determination using HPLC (capacity factor k/ calculated log*k*)

A Waters Alliance 2695 XE HPLC separation module and a Waters Photodiode Array Detector 2996 (Waters Corp., Milford, MA, USA) were used. A Symmetry® C_{18} 5 μm, 4.6 × 250 mm, Part No. WAT054275 (Waters Corp., Milford, MA, USA) chromatographic column was used. The HPLC separation process was monitored by Empower™ 2 Chromatography Data Software, Waters 2009 (Waters Corp., Milford, MA, USA). A mixture of MeOH p.a. (70%) and H_2O-HPLC—Mili-Q Grade (30%) was used as a mobile phase. The total flow of the column was 1.0 mL/min, injection volume, 30 μL, column temperature, 30 °C, and sample temperature, 10 °C. The detection wavelength of 210 nm was chosen. The KI methanolic solution was used for the dead time (t_D) determination. Retention times (t_R) were measured in minutes. The capacity factors k were calculated using the Empower™ 2 Chromatography Data Software according to formula $k = (t_R - t_D)/t_D$, where t_R is the retention time of the solute, whereas t_D denotes the dead time obtained using an unretained analyte. Log$k$, calculated from the capacity factor k, is used as the lipophilicity index converted to log$P$ scale. The log$k$ values of the individual compounds are shown in Table 1.

4.3. Lipophilicity calculations

Log$P$, that is the logarithm of the partition coefficient for *n*-octanol/water, was calculated using the programs CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) and ACD/LogP ver. 1.0 (Advanced Chemistry Development Inc., Toronto, Canada). *C*log$P$ values (the logarithm of *n*-octanol/water partition coefficient based on established chemical interactions) were generated by means of CS ChemOffice Ultra ver. 10.0 (CambridgeSoft, Cambridge, MA, USA) software. The results are shown in Table 1.

4.4. Study of inhibition photosynthetic electron transport (PET) in spinach chloroplasts

Chloroplasts were prepared from spinach (*Spinacia oleracea* L.) according to Masarovicova and Kralova.[47] The inhibition of photosynthetic electron transport (PET) in spinach chloroplasts was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA), using an artificial electron acceptor 2,6-dichlorophenol-indophenol (DCIPP) according to Kralova et al.,[40] and the rate of photosynthetic electron transport was monitored as photoreduction of DCPIP. The measurements were carried out in phosphate buffer (0.02 mol/L, pH 7.2) containing sucrose (0.4 mol/L), $MgCl_2$ (0.005 mol/L) and NaCl (0.015 mol/L). The chlorophyll content was 30 mg/L in these experiments, and the samples were irradiated (~100 W/m^2 with 10 cm distance) with a halogen lamp (250 W) using a 4 cm water filter to prevent warming of the samples (suspension temperature 22 °C). The studied compounds were dissolved in DMSO due to their limited water solubility. The applied DMSO concentration (up to 4%) did not affect the photochemical activity in spinach chloroplasts. The inhibitory efficiency of the studied compounds was expressed by IC_{50} values, that is, by molar concentration of the compounds causing 50% decrease in the oxygen evolution rate relative to the untreated control. The comparable IC_{50} value for a selective herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea, DCMU (Diurone®) was about 1.9 μmol/L. The results are summarized in Table 2.

The emission fluorescence spectra were recorded on a fluorescence spectrophotometer F-2000 (Hitachi, Tokyo, Japan) at room temperature (24 °C). The samples of chloroplast suspension (10 mg chlorophyll/L) with and without the studied inhibitor were excited at 436 nm using a slit width of 10 nm and were kept in the dark for10 min prior to the measurement. Due to low aqueous solubility the compounds were added to a chloroplast suspension in DMSO solution. The DMSO concentration in all samples was the same as in the control (10%).

4.5. In vitro antifungal susceptibility testing

The broth microdilution test[48,49] was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859, *C. krusei* E28, *Trichosporon asahii* 1188 and *Trichophyton mentagrophytes* 445. Fluconazole (FLU) was used as a standard, since it is a clinically used antimycotic drug. The procedure was performed with a twofold dilution of the compounds in RPMI 1640 (Sevapharma a.s., Prague, Czech Republic) buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulfonic acid (MOPS, Sigma, Germany). The final concentrations of the compounds ranged from 500 to 0.975 μmol. Drug-free controls were included. The MIC determination was performed according to the CLSI reference protocol M27-A2 for yeasts (IC_{80} value) and M38-A for moulds (IC_{50} value). IC_{50} values were defined as a 50% reduction of growth in comparison with the control, and IC_{80} values were defined as an 80% reduction of growth in comparison with the control. The trays were incubated at 35 °C, and MICs were read visually for filamentous fungi and photometrically for yeasts as absorbance at 540 nm after 24 h and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h. MICs were determined twice and in duplicate. The results are summarized in Table 2.

4.6. In vitro antibacterial susceptibility testing

The synthesized compounds were evaluated for in vitro antibacterial activity against methicillin-resistant *Staphylococcus aureus* H 5996/08 and *Staphylococcus epidermidis* H 6966/08. Phenoxymethylpenicillin (PMP) was used as the standard since it is a clinically used antibacterial drug. All strains were sub-cultured on nutrient agar (HiMedia) and maintained on the same medium at 4 °C. Prior to testing, each strain was passaged onto nutrient agar, and bacterial inocula were prepared by suspending a small portion of bacterial colony in sterile 0.85% saline. The cell density was adjusted to 0.5 McFarland units using a densitometer (Densi-La-Meter, PLIVA Lachema Diagnostika, Czech Republic). The final inoculum was made by 1:20 dilution of the suspension with the test medium (Mueller-Hinton broth). The compounds were dissolved in DMSO, and the anti-bacterial activity was determined using Mueller-Hinton broth (MH broth, HiMedia, pH 7.0 ± 0.2). Controls consisted of MH broth and DMSO alone. The final concentration of DMSO in the MH broth did not exceed 1% (v/v) of the total solution composition. The activity of the studied compounds was determined as the minimal inhibition concentration (MIC) according to NCCLS guidelines[50] using broth microdilution test. The MICs were defined as 90% inhibition of bacterial growth compared to the control and were determined after 24 and 48 h of static incubation at 37 °C. After incubation MICs were read visually as an absorbance at 540 nm. The results are shown in Table 2.

4.7. In vitro antimycobacterial evaluation

Mycobacterium kansasii CIT11/06 were grown in Middlebrook broth (MB), supplemented with Oleic-Albumin-Dextrose-Catalase supplement (OADC, Becton Dickinson, UK). Identification of these isolates was performed using biochemical and molecular protocols. At log phase growth, culture (10 mL) was centrifuged at 15,000 rpm/20 min using a bench top centrifuge (Model CR 4-12, Jouan Inc., UK). Following removal of the supernatant, the pellet was washed in fresh Middlebrook 7H9GC broth and re-suspended

in fresh supplemented MB (10 mL). The turbidity was adjusted to match McFarland standard No. 1 (3×10^8 cfu) with MB broth. A further 1:20 dilution of the culture was then performed in MB broth.

The antimicrobial susceptibility of all four mycobacterial species was investigated in a 96-well plate format. In these experiments, sterile deionised water (300 μL) was added to all outer-perimeter wells of the plates to minimize evaporation of the medium in the test wells during incubation. Each evaluated compound (100 μL) was incubated with each of the mycobacterial species (100 μL). Dilutions of each compound were prepared in duplicate. For all synthesized compounds, final concentrations ranged from 1000 μg/mL to 8 μg/mL. All compounds were prepared in DMSO, and subsequent dilutions were made in supplemented MB. The plates were sealed with parafilm and incubated at 37 °C for 5 days. Following incubation, a 10% addition of alamarBlue (AbD Serotec) was mixed into each well and readings at 570 nm and 600 nm were taken, initially for background subtraction and subsequently after 24 h re-incubation. The background subtraction is necessary for strongly coloured compounds, where the colour may interfere with the interpretation of any colour change. For non-interfering compounds, a blue colour in the well was interpreted as an absence of growth and a pink colour was scored as growth. The MIC was initially defined as the lowest concentration, which prevented a visual colour change from blue to pink. Isoniazid (INH) and pyrazinamide (PZA) were used as the standards as they are clinically used antimycobacterial drugs. The MIC for mycobacteria was defined as a 90% or greater (IC_{90}) reduction of growth in comparison with the control. The MIC/IC_{90} value is routinely and widely used in bacterial assays and is a standard detection limit according to the Clinical and Laboratory Standards Institute (CLSI, www.clsi.org). The results are summarized in Table 2.

4.8. In vitro cytotoxicity assay

Human monocytic leukemia THP-1 cells were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK; Methods of characterization: DNA Fingerprinting (Multilocus probes) and isoenzyme analysis). These cells were routinely cultured in RPMI 1640 (Lonza, Verviers, Belgium) medium supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich, St. Louis, MO, USA), 2% L-glutamine, 1% penicillin and streptomycin (Lonza) at 37 °C with 5% CO_2. Cells were passaged at approximately 1 week intervals. Cells were routinely tested for the absence of mycoplasma (Hoechst 33258 staining method). The tested compounds were dissolved in DMSO (Sigma–Aldrich) and added in five increasing concentrations to the cell suspension in the culture medium. The maximum concentration of DMSO in the assays never exceeded 0.1%. Subsequently, the cells were incubated for 24 h at 37 °C with 5% CO_2 to various compound concentrations ranging from 1 to 30 μM in RPMI 1640 medium. Cell toxicity was determined using a Cytotoxicity Detection Kit^{PLUS} Lactate dehydrogenase (LDH) assay kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. For LDH assays, cells were seeded into 96-well plates (5×10^4 cells·well^{-1} in 100 μL culture medium) in triplicate in serum-free RPMI 1640 medium, and measurements at 492 nm wavelength (Synergy 2 Multi-Mode Microplate Reader, BioTek, Vermont, USA) were taken 24 h after the treatment with tested compounds. The median lethal dose values, LD_{50}, were deduced through the production of a dose-response curve. All data were evaluated using GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). The results are summarized in Table 2.

LDH Test Principle: The LDH activity is determined by a coupled enzymatic reaction, whereby the tetrazolium salt INT is reduced to formazan. An increase in the amount of dead or plasma membrane damaged cells results in an increase of LDH enzyme activity in the culture supernatant. This increase in the amount of enzyme activity in the supernatant directly correlates to the amount of formazan formed during a limited time period. The formazan dye formed is water soluble and shows a broad absorption maximum at approximately 500 nm.

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