

# Synthesis of imidazoline and imidazo[2,1-*c*][1,2,4]triazole aryl derivatives containing the methylthio group as possible antibacterial agents

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**Abstract**—1-Arylimidazolidine-2-thiones (**1a–g**) were synthesized by the condensation reaction of *N*-arylethylenediamines with carbon disulfide in xylene medium. Their further alkylation with methyl iodide led to the formation of some biologically active 1-aryl-2-methylthio-imidazolines (**2a–g**). The 7-(4-methylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (**4b**) was obtained by the alkylation of the respective 7-(4-methylphenyl)-2,5,6,7-tetrahydroimidazo[2,1-*c*][1,2,4]triazol-3(*H*)-thione (**3b**) with methyl iodide. Antimicrobial activities of 1-aryl-2-methylthio-imidazolines (**2a–g**) and the 7-(4-methylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (**4b**) are presented. All tested compounds showed MIC in the range of 11.0–89.2 μM. Compounds **2a,e** were found to be equipotent to chloramphenicol in vitro, whereas **2a,c,e–g** and **4b** showed superior activity (MIC) to ampicillin. © 2006 Elsevier Ltd. All rights reserved.

## 1. Introduction

Pharmacophores are defined as specific spatial maps of structural properties common to compounds, which exhibit particular pharmacological activities. The idea of pharmacophores forms a new direction of pharmaceutical thinking, as substances from different groups of the system of organic chemistry may contain the same pharmacophore. The present paper is based on this idea.

Antimycobacterial activity of the compounds containing the alkylthio group bound to an electron-deficient carbon atom is already well known. For instance, Ditophal (*S,S'*-diethyl-dithioisophthalate), containing this structural moiety, found application in the treatment of lepromatous leprosy.<sup>1–3</sup>

It has been shown that the alkylthio group bound to an electron-deficient carbon atom in various heterocycles could be considered to be the pharmacophore of antimycobacterial and/or antimycotic activity. This hypothesis was formulated and proved by Klimešova et al.<sup>4–6</sup> and Waisser et al.<sup>7,8</sup> for 6-amino-2-alkylthiobenzothiazoles, 1-aryl-5-alkylthio-1,2,3,4-tetrazoles, substituted 4-alkylthiopyridine-4-carbothioamides and 4-carbonitriles and substituted 2-alkylthiopyridine-4-carbothioamides and 4-carbonitriles, bioactive molecules from different heterocyclic systems but with the same pharmacophore.

Moreover, on the other hand from the literature data it follows that depending on the type of substituent certain derivatives of imidazoline may also show antimicrobial properties.<sup>9–11</sup> The definite derivatives of imidazoline, aromatic diimidazolines related to pentamidine, were proved to be active against AIDS related opportunistic pathogens, such as *Candida albicans* and *Candida neoformans*.<sup>9</sup>

Previous studies concerning bridgehead nitrogen-heterocyclic compounds obtained by fusion of 4,5-dihydroimi-

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dazole and 1,2,4-triazole nuclei carried out in the Department of Synthesis and Chemical Technology of Pharmaceutical Substances have identified one compound having a sulfanyl group at position 3 and a 4-chlorophenyl substituent at position 7 (i.e., 7-(4-chlorophenyl)-3-thio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole) with a significant antifungal activity.<sup>12</sup> It is of interest that some derivatives having the same heterocyclic skeleton—7-aryl-5-methyl-3-thio-imidazo[2,1-*c*][1,2,4]triazol-6-ones described in the literature have been evaluated for their general pharmacological activities and have been found to possess high antifungal activity. These compounds exhibited fungicidal action almost equivalent to that of mancozeb (Dithane M-45) at 1000 ppm concentration and inhibited growth of *Aspergillus niger* and *Fusarium oxysporum* by more than 48% and 47% even at 10 ppm concentration.<sup>13</sup>

The following antifungal 1,2,4-triazole derivatives are applicable in medicine: fluconazole, itraconazole and terconazole. Moreover, from the literature data, it follows that depending on the type of substituent derivatives of 1,2,4-triazole show antimicrobial properties.<sup>14–17</sup>

Prompted by these reports and in attempt to prepare heterocyclic compounds of biological interest,<sup>12,18</sup> it seemed worthwhile to synthesize some of the hitherto unknown derivatives of 1-aryl-imidazoline and 7-aryl-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole containing the methylthio group as an expected pharmacophore of antimicrobial activity. In the present communication, we would like to report the results on the antimicrobial activity investigation of seven representatives of 1-aryl-2-methylthio-imidazolines (**2a–g**) and one imidazo-triazole derivative, that is, 7-(4-methylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (**4b**). For the estimation of potential activity in vitro the influence of tested compounds in examined concentrations of 100 and 200 µg mL<sup>−1</sup> on the growth of Gram-positive and Gram-negative bacterial strains, mould and yeast-like fungi strains and the MIC values against two reference strains of Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922) bacteria were determined.

## 2. Chemistry

The synthetic pathway for compounds described was achieved by a sequence of reactions starting from the respective anilines and is illustrated in Scheme 1.

In the first step, commercially available anilines were converted into *N*-arylethylenediamines by the Lehmann method<sup>19</sup> or by the classical Knoevenagel and Mercklin method with Takeda modification.<sup>20,21</sup> Their further condensation with carbon disulfide in the xylene medium led to the formation of intermediates—dithiocarbaminic acid derivatives, which could easily be cyclized in boiling solvent under reaction conditions to the 1-arylimidazolidine-2-thiones (compounds **1a,c–g**) with concomitant liberation of a hydrogen sulfide molecule. Alkylation of compounds

**1a,c–g** with 1 equiv methyl iodide afforded the 1-aryl-2-methylthio-imidazolines (**2a,c–g**) in 75–85% yields. Use of the methyl iodide over dimethyl sulfate for S-methylation was experimentally found to be superior in terms of yield. Biologically active 1-aryl-2-methylthio-imidazolines (**2a,c–g**) and their starting materials (**1a,c–g**) were prepared by patent pending according to Sztanke. The majority of obtained compounds, with the exception of **1a**, **2a,g**<sup>22–24</sup> and **1b**, **2b**,<sup>25</sup> are new and their synthesis, physicochemical, spectral and biological activity data have not been reported in the literature as yet. The 7-(4-methylphenyl)-3-methylthio-5*H*-6,7-dihydroimidazo[2,1-*c*][1,2,4]triazole (**4b**) was obtained in good yield (78%) from the respective 7-(4-methylphenyl)-2,5,6,7-tetrahydroimidazo[2,1-*c*][1,2,4]triazol-3(*H*)-thione<sup>25</sup> (**3b**) by alkylation with methyl iodide. The methylation reaction was carried out in ambient temperature for 24 h and then in boiling solvent for 5 h. Substrates were solved in anhydrous ethanol prior to mixing them together in a molar ratio of 1:1. The reaction conditions were established experimentally.

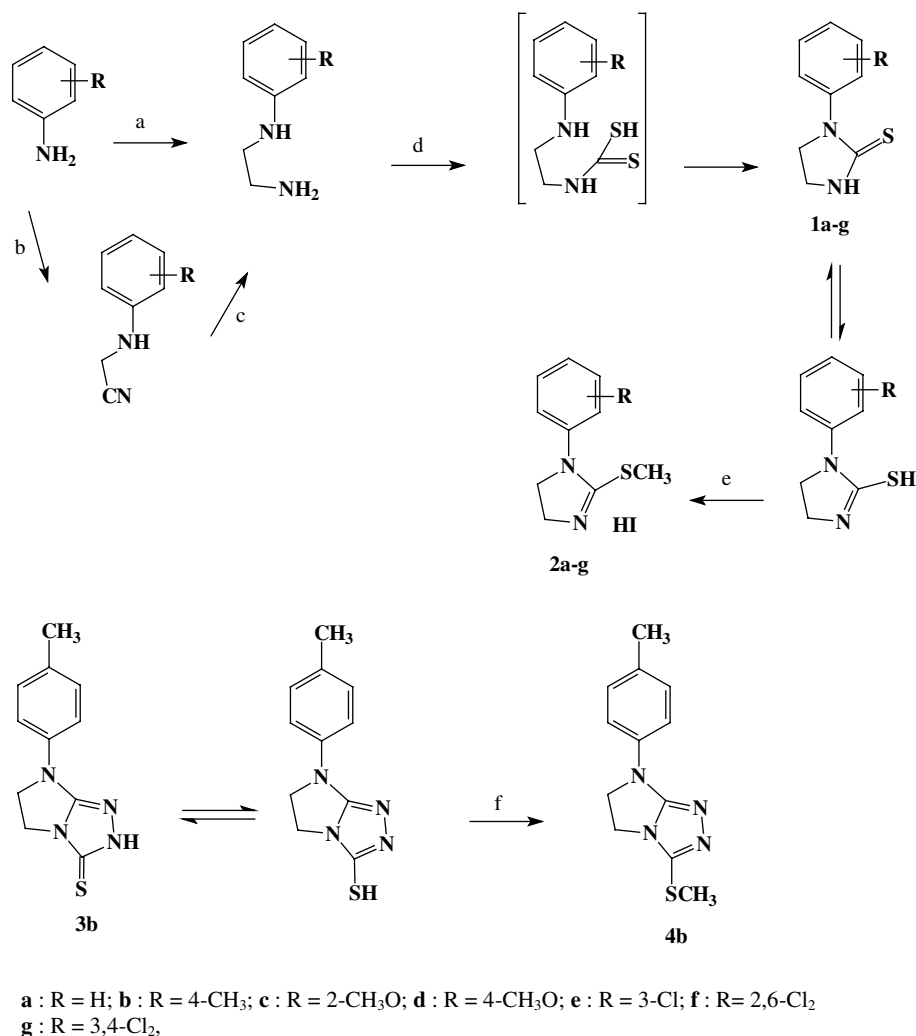
In view of continuous and widespread interest in the design and synthesis of novel heterocyclic derivatives containing the methylthio group as an expected pharmacophore, the synthetic approach leading to the formation of methylthio derivatives of imidazoline and imidazo-triazole, described by us, might be considered as a useful method for preparation of these biologically active compounds because of the affordability of the starting materials, good yields obtained and straightforward product isolation.

All new compounds reported here were characterized on the basis of complementary spectroscopic (<sup>1</sup>H NMR, IR and MS) and analytical data. The physicochemical properties and spectral data of synthesized compounds are presented in Table 1.

In the case of compounds **1a–g**, the existence of thiol–thione tautomerism is possible. In their <sup>1</sup>H NMR spectra, a slightly broadened singlet signal in the range 9.14–9.58 ppm and corresponding to the –NH–C=S group was observed. From this signal chemical shift value it can be concluded that the tautomeric equilibrium in solution should be moved towards 2-thione form rather than the 2-thiol one.

In the <sup>1</sup>H NMR spectra of compounds **2a–g**, a single absorption band at about 2.9 ppm corresponding to the S–CH<sub>3</sub> group and a single absorption band at about 9.9 ppm, characterizing the presence of N<sup>+</sup>H, were seen, while the signals belonging to –NH–C=S group disappeared.

NMR spectral characteristic of representative of the heterocyclic dihydroimidazo-triazole system (**4b**) revealed in its <sup>1</sup>H NMR spectrum the two double-doublet signals of the H5 and H6 at 4.21 and 4.51 ppm, with the coupling constants of *J* = 7.4 Hz, *J'* = 6.1 Hz and *J* = 7.5 Hz, *J'* = 6.1 Hz, respectively. The difference between chemical shifts



**Scheme 1.** Synthetic route to obtained compounds. Reagents and conditions: (a) aziridine, AlCl<sub>3</sub>, dry toluene; (b) HCHO, Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, NaCN, water, reflux; (c) H<sub>2</sub>, NiRa, methanol/NH<sub>3</sub>, 100 °C; (d) CS<sub>2</sub>, xylene, rt, 20 min, reflux, 7 h; (e) CH<sub>3</sub>I/MeOH, rt, 48 h, reflux, 6 h; (f) CH<sub>3</sub>I, abs EtOH, rt, 24 h, reflux, 5 h, Na<sub>2</sub>CO<sub>3</sub>.

of both signals can suggest an important difference in the acidity of both hydrogen atoms. Moreover, the two additional singlet signals derived from the CH<sub>3</sub> and the SCH<sub>3</sub> groups were observed at 2.28 and 2.89 ppm, integrating for three proton and three proton, respectively.

It is interesting to note that compounds **1a–g** are present in the solid state in the C=S form as indicated by their IR spectra showing the presence of two absorption bands at 1268–1284 and 1470–1479 cm<sup>-1</sup> and the absence of absorption in the region 2500–2650 cm<sup>-1</sup> for SH stretching.

The compounds **2a–g** and **4b** could be recognized by the presence of specific absorption band of the S–CH<sub>3</sub> bond in the range of 1309–1325 cm<sup>-1</sup>. The C=N absorption band was present in the IR spectra of compounds **2a–g** and **4b** in the region 1548–1561 cm<sup>-1</sup>.

The mass spectra of obtained compounds showed molecular ion peaks which were consistent with their molecular formulae.

### 3. Pharmacology

Determination of in vitro antimicrobial activity of the compounds tested was performed using the microdilution method, according to the National Committee for Clinical Laboratory Standards (NCCLS)<sup>26–28</sup> and the disc-diffusion method by Kirby–Bauer.<sup>29–31</sup>

The in vitro activities of eight compounds (**2a–g** and **4b**) against pathogenic bacteria, yeast-like fungi and moulds were compared. The following microorganisms were used: *S. aureus* ATCC 25923, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus agalactiae* (Gram-positive bacteria), *E. coli* ATCC 25922, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* (Gram-negative bacteria), *C. albicans* and *Aspergillus* spp. The majority of strains under study were clinical isolates, identified with conventional morphological and biochemical methods. The microdilution method for estimation of MIC value (the lowest concentration of compound required to inhibit the growth of the tested microorganism) was applied to

**Table 1.** Physicochemical and spectral properties of the synthesized compounds

Compound	R	Formula, mw	Mp (°C)	Yield (%)	IR (cm <sup>-1</sup> )	<sup>1</sup> H NMR (CDCl <sub>3</sub> as a solvent), <sup>a</sup> δ (ppm); EIMS: <i>m/z</i>
<b>1a</b>	H	C <sub>9</sub> H <sub>10</sub> N <sub>2</sub> S, 178.26	170–2	71	1275, 1474 >C=S	3.75–4.2 (m, 4H, 2CH <sub>2</sub> ); 7.02–7.65 (m, 5H, arom.) 9.22 (br s, 1H, NH); <i>m/z</i> : 178 [M <sup>+</sup> ]
<b>1b</b>	4-CH <sub>3</sub>	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> S, 192.28	Ref. 25	—	—	—
<b>1c</b>	2-CH <sub>3</sub> O	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> OS, 208.28	243–4	67	1280, 1476 >C=S	3.83 (s, 3H, OCH <sub>3</sub> ); 3.75–4.17 (m, 4H, 2CH <sub>2</sub> ); 7.15–7.70 (m, 4H, arom.); 9.14 (br s, 1H, NH); <i>m/z</i> : 208 [M <sup>+</sup> ]
<b>1d</b>	4-CH <sub>3</sub> O	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> OS, 208.28	156–8	65	1284, 1479 >C=S	3.84 (s, 3H, OCH <sub>3</sub> ); 3.67–4.19 (m, 4H, 2CH <sub>2</sub> ); 6.86–7.49 (m, 4H arom.); 9.21 (br s, 1H, NH); <i>m/z</i> : 208 [M <sup>+</sup> ]
<b>1e</b>	3-Cl	C <sub>9</sub> H <sub>9</sub> ClN <sub>2</sub> S, 212.70	181–3	67	1276, 1476 >C=S	3.74–4.3 (m, 4H, 2CH <sub>2</sub> ); 7.26–7.50 (m, 4H, arom.); 9.51 (br s, 1H, NH); <i>m/z</i> : 212 [M <sup>+</sup> ]
<b>1f</b>	2,6-Cl <sub>2</sub>	C <sub>9</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>2</sub> S, 247.15	226–8	64	1272, 1472 >C=S	3.86–4.15 (m, 4H, 2CH <sub>2</sub> ); 7.21–7.51 (m, 3H, arom.); 9.31 (br s, 1H, NH); <i>m/z</i> : 247 [M <sup>+</sup> ]
<b>1g</b>	3,4-Cl <sub>2</sub>	C <sub>9</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>2</sub> S, 247.15	165–7	62	1268, 1470 >C=S	3.88–4.22 (m, 4H, 2CH <sub>2</sub> ); 7.33–7.64 (m, 3H, arom.); 9.58 (br s, 1H, NH); <i>m/z</i> : 247 [M <sup>+</sup> ]
<b>2a</b>	H	C <sub>10</sub> H <sub>13</sub> IN <sub>2</sub> S, 320.20	163–5	82	1312 S–CH <sub>3</sub> 1511 N <sup>+</sup> H 1550 C=N	2.84 (s, 3H, SCH <sub>3</sub> ); 4.08–4.28 (m, 4H, 2CH <sub>2</sub> ); 7.15–7.74 (m, 5H, arom.); 9.85 (s, N <sup>+</sup> H); <i>m/z</i> : 320 [M <sup>+</sup> ]
<b>2b</b>	4-CH <sub>3</sub>	C <sub>11</sub> H <sub>15</sub> IN <sub>2</sub> S, 334.22	Ref. 25	—	—	—
<b>2c</b>	2-CH <sub>3</sub> O	C <sub>11</sub> H <sub>15</sub> IN <sub>2</sub> OS, 350.22	181–3	78	1322 S–CH <sub>3</sub> 1507 N <sup>+</sup> H 1556 C=N	2.93 (s, 3H, SCH <sub>3</sub> ); 3.83 (s, 3H, OCH <sub>3</sub> ); 4.24–4.38 (m, 4H, 2CH <sub>2</sub> ); 7.18–7.79 (m, 4H, arom.); 9.85 (s, N <sup>+</sup> H); <i>m/z</i> : 350 [M <sup>+</sup> ]
<b>2d</b>	4-CH <sub>3</sub> O	C <sub>11</sub> H <sub>15</sub> IN <sub>2</sub> OS, 350.22	165–7	79	1325 S–CH <sub>3</sub> 1505 N <sup>+</sup> H 1557 C=N	2.95 (s, 3H, SCH <sub>3</sub> ); 3.84 (s, 3H, OCH <sub>3</sub> ); 4.31–4.42 (m, 4H, 2CH <sub>2</sub> ); 6.93–7.41 (m, 4H, arom.); 9.9 (s, N <sup>+</sup> H); <i>m/z</i> : 350 [M <sup>+</sup> ]
<b>2e</b>	3-Cl	C <sub>10</sub> H <sub>12</sub> ClIN <sub>2</sub> S, 354.64	175–7	85	1310 S–CH <sub>3</sub> 1510 N <sup>+</sup> H 1551 C=N	2.89 (s, 3H, SCH <sub>3</sub> ); 4.28–4.44 (m, 4H, 2CH <sub>2</sub> ); 7.19–7.56 (m, 4H, arom.); 9.70 (s, N <sup>+</sup> H); <i>m/z</i> : 354 [M <sup>+</sup> ]
<b>2f</b>	2,6-Cl <sub>2</sub>	C <sub>10</sub> H <sub>11</sub> Cl <sub>2</sub> IN <sub>2</sub> S, 389.09	164–6	79	1314 S–CH <sub>3</sub> 1512 N <sup>+</sup> H 1561 C=N	2.91 (s, 3H, SCH <sub>3</sub> ); 4.31–4.47 (m, 4H, 2CH <sub>2</sub> ); 7.11–7.44 (m, 3H, arom.); 10.1 (s, N <sup>+</sup> H); <i>m/z</i> : 389 [M <sup>+</sup> ]
<b>2g</b>	3,4-Cl <sub>2</sub>	C <sub>10</sub> H <sub>11</sub> Cl <sub>2</sub> IN <sub>2</sub> S, 389.09	177–9	75	1316 S–CH <sub>3</sub> 1515 N <sup>+</sup> H 1557 C=N	2.96 (s, 3H, SCH <sub>3</sub> ); 4.17–4.32 (m, 4H, 2CH <sub>2</sub> ); 7.26–7.64 (m, 3H, arom.); 10.3 (s, N <sup>+</sup> H); <i>m/z</i> : 389 [M <sup>+</sup> ]
<b>3b</b>	4-CH <sub>3</sub>	C <sub>11</sub> H <sub>12</sub> N <sub>4</sub> S, 232.31	Ref. 25	—	—	—
<b>4b</b>	4-CH <sub>3</sub>	C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> S, 246.33	214–16	78	1309 S–CH <sub>3</sub> 1548 C=N	2.28 (s, 3H, CH <sub>3</sub> ); 2.89 (s, 3H, SCH <sub>3</sub> ); 4.21 ( <i>J</i> = 7.4 Hz, <i>J'</i> = 6.1 Hz, dd, 2H, CH <sub>2</sub> ); 4.51 ( <i>J</i> = 7.5 Hz, <i>J'</i> = 6.1 Hz, dd, 2H, CH <sub>2</sub> ); 7.11–7.55 (m, 4H, arom.); <i>m/z</i> : 246 [M <sup>+</sup> ]

<sup>a</sup> Except for compound **4b**: DMSO-*d*<sub>6</sub>.

evaluate the antibacterial activity. In this method, two reference strains of bacteria—*S. aureus* ATCC 25923 and *E. coli* ATCC 25922, were included in this study. The antibacterial potency of the compounds under conditions was compared with the activity of topical antibacterial drugs—ampicillin and chloramphenicol.

#### 4. Results and discussion

The antimicrobial activities of obtained compounds against bacterial, moulds and yeast-like fungi strains and the MIC values against two reference strains (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922) were tested by using the disc-diffusion and the microdilution assays. The results from experiments were compared with those of ampicillin and chloramphenicol as the standards for antibacterial agents. The minimum inhibitory concentrations (MICs) of ampicillin and chloramphenicol for *S. aureus* and *E. coli* are 35.8, 35.8  $\mu\text{M}$  and 12.1, 12.1  $\mu\text{M}$ , respectively. MIC values of the examined compounds are listed in Table 3.

Eight compounds tested in the present study were found to have highly significant antibacterial activities against the microorganisms listed in Tables 2 and 3, but no antifungal activities. All examined compounds were also inactive against moulds and yeast-like fungi. 1-Aryl-2-methylthio-imidazolines (**2a–g**) showed high activity in

relation to typical Gram-positive (*S. aureus* ATCC 25923) and Gram-negative (*E. coli* ATCC 25922) bacterial strains with MIC values from 11.0 to 89.2  $\mu\text{M}$ . On the whole, the most potent in vitro antibacterial effect was demonstrated by compounds **2e** and **2a** in relation to typical Gram-positive *S. aureus* ATCC 25923 reference bacterial strain and Gram-negative *E. coli* ATCC 25922 standard bacterial strain, with minimal inhibitory concentration values of 11.0 and 12.2  $\mu\text{M}$ , respectively. These derivatives were equipotent to chloramphenicol and 3-fold more potent to ampicillin (Table 3). Compound **2a** was also found to inhibit growth of *P. vulgaris* and *S. epidermidis* in a concentrations of 100  $\mu\text{g mL}^{-1}$  (312.3  $\mu\text{M}$ ) and 200  $\mu\text{g mL}^{-1}$  (624.6  $\mu\text{M}$ ), *K. pneumoniae* and *E. aerogenes* in a concentration of 200  $\mu\text{g mL}^{-1}$  (624.6  $\mu\text{M}$ ) in the disc-diffusion assay. The growth of *S. aureus* ATCC 25923 was inhibited by compound **2e**, that is, 1-(3-chlorophenyl)-2-methylthio-imidazoline hydroiodide at 11.0  $\mu\text{M}$  and its derivative **2d** (1-(4-methoxyphenyl)-2-methylthio-imidazoline hydroiodide) at 89.2  $\mu\text{M}$ . It is noteworthy in this case that the replacement of 3-chlorophenyl substituent in **2e** with 4-methoxy substituent as in **2d** led to 8-fold decrease in activity against *S. aureus* ATCC 25923. Compound **2e** also significantly inhibited growth of *S. epidermidis* in a concentration of 200  $\mu\text{g mL}^{-1}$  (564.0  $\mu\text{M}$ ). This bacterial strain was found to be intermediate susceptible to that compound in a concentration of 100  $\mu\text{g mL}^{-1}$  (282.0  $\mu\text{M}$ ). Compound **2d** showed in vitro effectiveness against *P. aeruginosa* in a concentration of 200  $\mu\text{g mL}^{-1}$

**Table 2.** Antimicrobial activities of eight evaluated compounds against the tested bacterial and fungal isolates using the disc-diffusion method

Microorganisms	Compound																Standard
	2a		2b		2c		2d		2e		2f		2g		4b		
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	
<i>Escherichia coli</i> (ATCC)	++	++	+	+	+	++	–	–	–	–	+	++	+	++	–	–	++
<i>Pseudomonas aeruginosa</i>	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
<i>Proteus vulgaris</i>	++	++	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–
<i>Klebsiella pneumoniae</i>	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Enterobacter aerogenes</i>	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Staphylococcus aureus</i> (ATCC)	+	+	–	–	+	++	–	+	++	+++	+	++	+	++	+	++	++
<i>Staphylococcus epidermidis</i>	+	+	–	–	–	–	+	++	+	++	+	+	+	+	+	+	+
<i>Streptococcus pyogenes</i>	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	++
<i>Streptococcus agalactiae</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++
<i>Candida albicans</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Aspergillus</i> spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Zones of growth inhibition in: –, +, ++, +++.

–: 0–10 mm (R, resistant); +: 11–16 mm (I, intermediate susceptible); ++: 17–25 mm (S, susceptible); +++: >25 mm (S, susceptible); Standard: ampicillin in a concentration of 200  $\mu\text{g mL}^{-1}$  (572.4  $\mu\text{M}$ ).

I: concentration of 100  $\mu\text{g mL}^{-1}$ , which correspond to concentration ranges of 257.0–406.0  $\mu\text{M}$  depending on molecular weight of the examined compound.

II: concentration of 200  $\mu\text{g mL}^{-1}$ , which correspond to concentration ranges of 514.0–812.0  $\mu\text{M}$  depending on molecular weight of the examined compound.

**Table 3.** Antibacterial activity expressed as MIC ( $\mu\text{M}$ ) of selected compounds

Microorganisms/code	Compound									
	2a	2b	2c	2d	2e	2f	2g	4b	A	C
<i>Escherichia coli</i> ATCC 25922	12.2	46.7	22.3	NT	NT	20.1	20.1	NT	35.8	12.1
<i>Staphylococcus aureus</i> ATCC 25923	NT	NT	22.3	89.2	11.0	20.1	20.1	31.7	35.8	12.1

NT: not tested due to inactivity in the disc-diffusion assay. Standards: A—ampicillin; C—chloramphenicol.



(571.1  $\mu\text{M}$ ) and against *P. vulgaris* and *S. epidermidis* in concentrations of 100  $\mu\text{g mL}^{-1}$  (285.5  $\mu\text{M}$ ) and 200  $\mu\text{g mL}^{-1}$  (571.1  $\mu\text{M}$ ) in the disc-diffusion assay. Both reference strains: *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were inhibited by compounds **2c,f** and **g** at MIC values of 22.3, 20.1 and 20.1  $\mu\text{M}$ , respectively. These derivatives were found to be 1.6- to 1.8-fold more potent than ampicillin and about 1.8 times less potent than chloramphenicol in vitro. Moreover, compounds **2f** and **g** were found to inhibit growth of *S. epidermidis* in concentrations of 100  $\mu\text{g mL}^{-1}$  (257.0  $\mu\text{M}$ ) and 200  $\mu\text{g mL}^{-1}$  (514.0  $\mu\text{M}$ ) in the disc-diffusion assay. The activity of **2f** was specific for *S. pyogenes*, as was found in the disc-diffusion assay. None of the tested compounds, with the exception of **2f**, was found to inhibit growth of those bacteria. *E. coli* ATCC 25922 was inhibited by compounds **2a** and **b** at 12.2 and 46.7  $\mu\text{M}$ , respectively. In this case, an introduction of methyl group in the position 4 to phenyl ring as in **2b** led to an almost 4-fold decrease in activity against *E. coli* ATCC 25922. Examined 7-(4-methylphenyl)-3-methylthio-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole (**4b**) was strongly active against *S. aureus* ATCC 25923, with MIC results at 31.7  $\mu\text{M}$ . Its antibacterial potency was hardly 1.1 times higher than that of ampicillin and 2.6-fold lower in comparison to chloramphenicol. This compound was also found to exhibit activity against *S. epidermidis* in concentrations of 100  $\mu\text{g mL}^{-1}$  (406.0  $\mu\text{M}$ ) and 200  $\mu\text{g mL}^{-1}$  (812.0  $\mu\text{M}$ ) in the disc-diffusion assay.

According to the results obtained, compounds **2a,e** were found to be equipotent to chloramphenicol and superior to ampicillin antibacterial in vitro activity with MIC values of 12.2 and 11.0  $\mu\text{M}$ , respectively, against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, respectively. Compounds **2a,c,e-g** and **4b** were found to be more potent than ampicillin in vitro.

Taking into account the significant antibacterial activities of the presented compounds, the research in this field will be continued. This refers to the structure modification of the presented molecules and synthesis of the analogous systems with alkylthio chain. The study thus represents a further confirmation of hypothesis of the alkylthio group bound to an electron-deficient carbon atom being a pharmacophore of antibacterial activity as the results fall well in line with it.

## 5. Conclusion

In this report, an easy and useful method to synthesize antibacterially active imidazoline and imidazo-triazole aryl derivatives containing the methylthio group as an expected pharmacophore has been presented. The identified analogues, in particular 1-aryl-2-methylthio-imidazoline derivatives (**2a** and **e**), more potent than ampicillin and equipotent to chloramphenicol, can serve as novel templates for bacterial infection chemotherapy. Further optimization of these identified chemical leads can possibly lead to more active molecules against bacterial infections. Since both of analogues (**2a** and **e**) are showing promising results, studies to establish their

in vivo efficacy and safety are being planned for their further development.

## 6. Experimental protocols

### 6.1. Instrumentations and general materials

Chemicals (carbon disulfide and methyl iodide) were purchased from Merck as 'synthesis grade' and used without further purification. Melting points (mp) were determined on a Boetius apparatus and are given uncorrected.  $^1\text{H}$  NMR spectra were recorded on a Tesla BS-567A 100 MHz spectrometer in  $\text{CDCl}_3$  (**1a-g** and **2a-g**) and on a Varian Gemini 200 MHz spectrometer in  $\text{DMSO}-d_6$  (**4b**) with TMS as an external standard at 295 K. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1725X spectrometer. Mass spectroscopic analyses were performed on an AMD-402 mass spectrometer for molecular ion peaks. Thin-layer chromatography was carried out on commercial Merck  $\text{SiO}_2$  60 F<sub>254</sub> plates having fluorescence indicator; the spots were visualized with UV light  $\lambda = 254$  nm and by spraying with a 2% ethanol solution of ninhydrin or charging reagent. Elemental analyses were performed on a Perkin-Elmer analyzer and were in the range of  $\pm 0.4\%$  for each element analyzed (C, H, N, Cl, I and S). More detailed data for compounds **1b**, **2b** and **3b** are given in Ref. 25.

**6.1.1. General procedure for synthesis of 1-arylimidazolidine-2-thiones (1a,c-g).** Appropriate *N*-arylethylenediamine (0.1 mol) was dissolved in 120 mL of xylene. Then carbon disulfide (0.1 mol) in xylene (30 mL) was added dropwise over the time of 20 min. During that time precipitation of the intermediate solid started. The reaction mixture was slowly brought to boil and refluxed for 7 h, next left overnight at room temperature. The crude product obtained upon cooling was filtered and finally purified by crystallization from methanol.

**6.1.2. General method for synthesis of 1-aryl-2-methylthio-imidazoline hydroiodides (2a,c-g).** Appropriate 1-arylimidazolidine-2-thione (0.1 mol) and methyl iodide (0.1 mol) in 50 mL of methanol were left at ambient temperature in closed flask for 48 h. Then the mixture was heated under reflux for 6 h. The solvent was removed by evaporation, oily residue was cooled and triturated with diethyl ether. The crude product was collected, washed with cold ethanol and finally crystallized from propan-2-ol.

**6.1.3. Synthesis of 7-(4-methylphenyl)-3-methylthio-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazole (4b).** 7-(4-Methylphenyl)-2,5,6,7-tetrahydroimidazo[2,1-c][1,2,4]triazol-3(*H*)-thione (0.05 mol)<sup>25</sup> and methyl iodide (0.05 mol) in 50 mL of absolute ethanol were allowed to stand at the ambient temperature for 24 h in closed flask. Then the mixture was heated under reflux for 5 h, cooled and left overnight. The solution was then evaporated to dryness, the residue taken up in water (50 mL), basified with solid sodium carbonate and the suspension was extracted with chloroform (3  $\times$  25 mL). Evaporation of the dried

chloroform extracts yielded a solid, which was heated under reflux with methanol (40 mL) for 1 h. The excess solvent was removed by evaporation and upon cooling the precipitate was collected, washed 2×20 mL cold propan-2-ol and finally recrystallized from methanol.

## 6.2. Microbiology

**6.2.1. Disc diffusion assay.** Assay of antimicrobial activity in vitro. The synthesized compounds were tested for their antimicrobial (antibacterial and antifungal) activities by disc-diffusion method by Kirby–Bauer, using Mueller–Hinton medium for bacteria and the same medium with 4% glucose for fungi. The majority of tested microorganisms were isolated from clinical specimens of the Laboratory of Medical Microbiology Department, Medical University of Lublin. The assayed collection included the following microorganisms: *S. epidermidis*, *S. pyogenes*, *S. agalactiae* (Gram-positive bacteria), *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae*, *E. aerogenes* (Gram-negative bacteria), *C. albicans* and *Aspergillus* spp. Besides, two reference strains of bacteria—*S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were included in these studies.

In the disc-diffusion method, sterile paper discs (Ø 5 mm) impregnated with compound dissolved in dimethylsulfoxide (DMSO) at concentrations of 100 and 200 µg mL<sup>-1</sup> were used. Discs containing DMSO were used as control. The microorganism cultures were spread over the following appropriate media: Mueller–Hinton agar for *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. agalactiae*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae*, *E. aerogenes* and Sabouraud's agar for the yeast-like fungi (*C. albicans*) and for the moulds (*Aspergillus* spp.) in Petri dishes. Then, the paper discs impregnated with the solutions of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35 °C/24 h for the microorganism cultures. After incubation, the growth inhibition zones around the discs were observed, indicating that the examined compound inhibits the growth of microorganism.<sup>25–27</sup> Each assay in this experiment was repeated three times. Ampicillin in a concentration of 200 µg mL<sup>-1</sup> (572.4 µM) was used as a standard drug. Dimethylsulfoxide was used as solvent control. Results were interpreted in terms of the diameter of the inhibition zone and are shown in Table 2.

**6.2.2. Microdilution assays.** The minimal inhibitory concentration (MIC) values for all compounds tested, defined as the lowest concentration of the compound preventing the visible growth, were determined by using the microdilution broth method according to NCCLS standards.<sup>26</sup> The inocula of microorganisms were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The test compounds dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (500 µg mL<sup>-1</sup>) to be tested. Then serial twofold dilutions were made in concentration ranges from 1.95 to 500 µg mL<sup>-1</sup> in 10 mL sterile tubes. A prepared suspension of the standard microorganisms was added to each dilution in a 1:1 ratio. Growth (or its lack) of microor-

ganisms was determined visually after incubation for 24 h at 37 °C. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC.

The minimal inhibitory concentration (MIC) values were studied for reference bacterial strains: *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 towards eight compounds determined in the disc-diffusion assay. Ampicillin and chloramphenicol were used as standard drugs for comparison in the antibacterial studies. Control experiments using dimethylsulfoxide were done for antibacterial activity studies. The presented results were obtained from three independent measurements. The MIC values (in micromolars) are given in Table 3. The investigations were carried out in the Department of Medical Microbiology, Medical University, Lublin.

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