



# Synthesis and antimicrobial activity of 3,4-bis(arylthio)maleimides

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## Abstract

A series of 3,4-bis(arylthio)maleimides were synthesized and their antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria, including multidrug resistant (MDR) strains and some fungi. Most compounds turned out to be highly active, activity being dependent on substituents on phenyl rings.

The spread of antibiotic-resistant bacteria poses a substantial threat with high morbidity and mortality worldwide. The discovery of new scaffolds derived by chemical synthesis could open the way to alternative classes of antimicrobial agents with new mechanism of action and activity against multidrug resistant (MDR) species of bacteria and fungi [1, 2]. Maleimide derivatives are known for their wide spectrum of biological activity [3–6]. The compounds of interest—3,4-bis(phenylthio)maleimides—are widely used by researchers for the purpose of incorporating fluorescent tags into proteins or other modifications of proteins and hormones, including binding them to a polymer [7–10].

Also bis-(alkylthio)maleimide derivatives have been prepared from teicoplanin pseudoaglycon by reaction of its primary amino group with *N*-ethoxycarbonyl bis-alkylthiomaleimides. Some of the new derivatives displayed excellent antibacterial activity against resistant bacteria [11]. Bis(benzylthio)maleimide derivatives of teicoplanin pseudoaglycon showed good activity against vancomycin- and teicoplanin-resistant enterococci [12]. Antimicrobial activity of 3-(arylthio)maleimide derivatives was reported [13], but antimicrobial activity of 3,4-bis(arylthio)maleimides was not studied before. In the present work such compounds are shown to be rather active antimicrobial agents, capable of overcoming bacterial MDR.

The symmetrical 3,4-bis(arylthio)maleimides **3a–g** were synthesized by the reaction of 3,4-dibromomaleimide with two equivalents of various thioaryl derivatives and triethylamine in THF (Scheme 1).

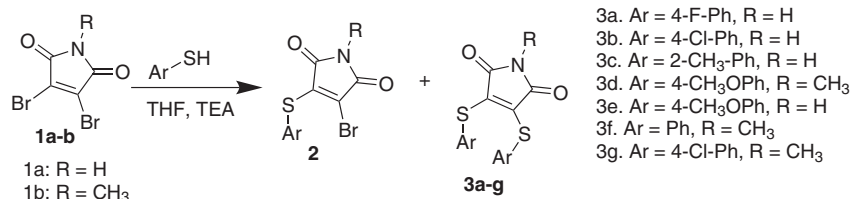
We failed to isolate monosubstituted products **2** even when 0.3 equivalents of thiophenol were used, carrying out the reaction at 0 °C. Changing the solvent to less polar (i.e. toluene) had no effect. We suggest that 3-bromo-4-(arylthio)maleimide intermediate **2** is much more reactive than dibromomaleimide **1**, resulting in a mixture of disubstituted product **3** and unreacted **1**.

All compounds **3a–g** were tested against Gram-positive and Gram-negative bacteria and fungi. The minimum inhibitory concentrations (MIC) for Gram-positive and Gram-negative bacteria were determined by the microdilution method in a cation-adjusted Müller–Hinton medium in accordance with the requirements of the Institute of Clinical and Laboratory Standards (CLSI/NCCLS) [14]. The activity of the test compounds against various cultures of yeast and mycelial fungi was estimated using the recommendation by CLSI/NCCLS micromethod [15, 16] by twofold serial dilutions in the nutrient medium RPMI 1640 (liquid, with L-glutamine and without sodium bicarbonate). Observed MIC are presented in the following Tables 1 and 2.

All compounds were active against Gram-positive *S. aureus*, *S. epidermidis* and *S. haemolyticus*. Compounds **3f** and **3g**, which both have an *N*-methyl group at the maleimide moiety, are generally less active against almost all strains, at the same time being more active against Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. However, compounds **3d** and **3e** show an opposite pattern. All compounds turned out to be less active than the reference compound levofloxacin on all but three bacterial

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**Scheme 1** Synthesis of 3,4-bis(arylthio)maleimides **3a-g****Table 1** MIC (μg/ml) against bacterial strains

Bacterial strains	3a	3b	3c	3d	3e	3f	3g	Levofloxacin
<i>Staphylococcus aureus</i> 25923 ATCC	4	1	0.5	4	4	4	2	0.25
<i>Staphylococcus aureus</i> 3798	2	0.5	1	4	2	4	2	32
<i>Staphylococcus aureus</i> 100KC	1	0.25	1	4	1	4	1	32
<i>Staphylococcus aureus</i> 700699 ATCC	2	1	2	4	2	4	2	16
<i>Staphylococcus aureus</i> 10	4	1	2	4	4	4	2	0.13
<i>Staphylococcus aureus</i> 5 (MRSA)	4	1	2	4	4	4	2	0.25
<i>Staphylococcus epidermidis</i> 533	4	1	1	8	4	2	1	0.5
<i>Staphylococcus haemolyticus</i> 585	4	1	1	4	4	4	2	0.5
<i>Acinetobacter baumannii</i> 5696	1	0.5	>64	>64	1	>64	>64	4
<i>Enterococcus faecium</i> 569	4	2	16	>64	4	>64	>64	1
<i>Escherichia coli</i> 25922 ATCC	16	16	>64	>64	16	4	2	0.06
<i>Klebsiella pneumoniae</i> 13883 ATCC	16	16	64	>64	16	4	2	0.25
<i>Proteus vulgaris</i> 13315 ATCC	16	16	>64	>64	16	>64	>64	4
<i>Salmonella choleraesuis</i> 14028 ATCC	16	16	>64	>64	16	>64	>64	0.13
<i>Pseudomonas aeruginosa</i> 27853 ATCC	8	8	>64	>64	8	4	4	1

strains. In comparison to 3-(arylthio)maleimide derivatives [13] our compounds show mostly similar level of activity, but wider spectrum of action.

Some compounds were also tested of fungi strains (Table 2). Even the most active compound **3g** is less active than the reference compound—amphotericin B.

All the reagents were obtained commercially and used without further purification. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin-layer chromatography using silica-gel 60 F<sub>254</sub>-coated Al plates (Merck) and spots were observed under UV light. <sup>1</sup>H NMR and <sup>13</sup>C NMR (in DMSO-*d*<sub>6</sub>) spectra were recorded on a Varian VXR-400 spectrometer at 400 MHz and 100 MHz, respectively; the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by ESI method on microTOF-QII (Bruker Daltonics GmbH).

General method for synthesizing 3,4-bis(arylthio)maleimides **3a-g**: To the solution of dibromomaleimide **1a** or *N*-methyldibromomaleimide **1b** (1 mmol) in THF (20 ml) was added solution of the thiophenol (2.2 mmol) and triethylamine (2.2 mmol) in one portion. The resulting solution was stirred at room temperature for 1 h, then evaporated in

vacuo and the residue was redissolved in ethyl acetate-water (20 + 20 ml) mixture. The organic layer was separated, washed with aq. NaHCO<sub>3</sub>, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was purified by flash chromatography (ethyl acetate: petroleum ether 3:1).

3,4-Bis((4-fluorophenyl)thio)-1*H*-pyrrole-2,5-dione (**3a**) was obtained as a yellow solid, yield 64%. Mp 145–146 °C (diethyl ether). <sup>1</sup>H NMR: δ 7.13 (4 H, t, *J* = 9.7 Hz), 7.29–7.32 (4 H, m), 11.37 (1 H, s, NH) <sup>13</sup>C NMR: δ 115.95, 116.18, 124.68, 133.25, 133.33, 135.71, 160.77, 163.21, 167.57. ESI-HRMS: calculated for C<sub>16</sub>H<sub>9</sub>F<sub>2</sub>NO<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup> 350.0116, found *m/z* 350.0107.

3,4-Bis((4-chlorophenyl)thio)-1*H*-pyrrole-2,5-dione (**3b**) was obtained as a yellow solid, yield 77%. Mp 165–166 °C (diethyl ether). <sup>1</sup>H NMR: δ 7.26 (4 H, d, *J* = 8.5 Hz), 7.32 (4 H, d, *J* = 8.5 Hz), 11.43 (1 H, s, NH) <sup>13</sup>C NMR: δ 128.20, 128.87, 132.32, 132.83, 135.80, 167.48. ESI-HRMS: calculated for C<sub>16</sub>H<sub>9</sub>Cl<sub>2</sub>NO<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup> 381.9525, found *m/z* 381.9542.

3,4-Bis(o-tolylthio)-1*H*-pyrrole-2,5-dione (**3c**) was obtained as a yellow solid, yield 50%. Mp 149–151 °C. <sup>1</sup>H NMR: δ 2.10 (6 H, s, CH<sub>3</sub>), 7.13 (10 H, m), 11.30 (1 H, s, NH). <sup>13</sup>C NMR: δ 20.52, 126.88, 128.79, 129.04, 130.72, 132.48, 136.26, 139.32, 168.07. ESI-HRMS:

**Table 2** MIC ( $\mu\text{g/ml}$ ) against fungi strains

Compound/ Organism	<i>Candida albicans</i> ATCC 14053	<i>Aspergillus niger</i> ATCC 16404	<i>Fusarium oxysporum</i> VKM F-140
<b>3a</b>	48	12	64
<b>3b</b>	32	64	$>64$
<b>3c</b>	64	16	$>64$
<b>3e</b>	64	4	48
<b>3g</b>	4	4	$>64$
<b>3d</b>	64	16	32
<b>Amphotericin B</b>	1	1	4

calculated for  $\text{C}_{18}\text{H}_{15}\text{NO}_2\text{S}_2$   $[\text{M} + \text{H}]^+$  342.0617, found  $m/z$  342.0612.

3,4-Bis((4-methoxyphenyl)thio)-1-methyl-1*H*-pyrrole-2,5-dione (**3d**) was obtained as a red amorphous solid, yield 72%.  $^1\text{H}$  NMR:  $\delta$  2.82 (3 H, s,  $\text{CH}_3$ ), 3.73 (6 H, s,  $\text{OCH}_3$ ), 6.86 (4 H, d,  $J = 8.9$  Hz), 7.26 (4 H, d,  $J = 8.9$  Hz).  $^{13}\text{C}$  NMR  $\delta$  24.80, 39.49, 39.70, 39.91, 40.12, 40.33, 55.76, 115.16, 119.93, 134.10, 136.05, 160.08, 166.85. ESI-HRMS: calculated for  $\text{C}_{19}\text{H}_{17}\text{NO}_4\text{S}_2$   $[\text{M} + \text{H}]^+$  388.0672, found  $m/z$  388.0726.

3,4-Bis((4-methoxyphenyl)thio)-1*H*-pyrrole-2,5-dione (**3e**) was obtained as a red amorphous solid, yield 65%.  $^1\text{H}$  NMR:  $\delta$  3.74 (6 H, s,  $\text{OCH}_3$ ), 6.87 (4 H, d,  $J = 8.8$  Hz), 7.26 (4 H, d,  $J = 8.8$  Hz).  $^{13}\text{C}$  NMR:  $\delta$  55.30, 114.70, 119.64, 133.58, 136.13, 159.58, 167.51. ESI-HRMS: calculated for  $\text{C}_{20}\text{H}_{19}\text{NO}_6\text{S}_2$   $[\text{M} + \text{H}]^+$  434.0727, found  $m/z$  434.0697.

1-Methyl-3,4-bis(phenylthio)-1*H*-pyrrole-2,5-dione (**3f**) was obtained as a yellow solid, yield 80%. Mp 105–106 °C.  $^1\text{H}$  NMR:  $\delta$  2.90 (3 H, s,  $\text{CH}_3$ ), 7.21–7.31 (10 H, m) NMR  $^{13}\text{C}$   $\delta$ : 24.53, 127.92, 129.02, 130.71, 135.81, 166.50. ESI-HRMS: calculated for  $\text{C}_{21}\text{H}_{21}\text{NO}_6\text{S}_2$   $[\text{M} + \text{H}]^+$  328.0460, found  $m/z$  328.0486.

3,4-Bis((4-chlorophenyl)thio)-1-methyl-1*H*-pyrrole-2,5-dione (**3g**) was obtained as a yellow solid, yield 77%. Mp 120–121 °C.  $^1\text{H}$  NMR:  $\delta$  2.88 (3 H, s,  $\text{CH}_3$ ), 7.32 (4 H, d,  $J = 8.5$  Hz), 7.26 (4 H, d,  $J = 8.5$  Hz). NMR  $^{13}\text{C}$   $\delta$ : 25.01, 128.56, 129.35, 132.77, 133.36, 135.80, 166.87. ESI-HRMS: calculated for  $\text{C}_{17}\text{H}_{11}\text{Cl}_2\text{NO}_2\text{S}_2$   $[\text{M} + \text{H}]^+$  395.9681, found  $m/z$  395.9662.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Tacconelli E, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. 2018;3:318–27.
- Butler MS, Blaskovich M, Cooper MA. Antibiotics in the clinical pipeline at the end of 2015. *J Antibiot*. 2017;70:3–24.
- Panov AA, Simonov AY, Lavrenov SN, Lakatos SA, Trenin AS. 3,4-Disubstituted maleimides: synthesis and biological activity. *Chem Heterocycl Compd*. 2018;54:103.
- Bakat A, et al. Cytotoxic effects of a novel maleimide derivative on epithelial and tumor cells. *Bioorg Chem*. 2017;72:199–207.
- van Eis MJ, et al. Indolyl-naphthyl-maleimides as potent and selective inhibitors of protein kinase C- $\alpha/\beta$ . *Bioorg Med Chem Lett*. 2017;27:781–6.
- Yuan X, et al. Synthesis and evaluation of 1*H*-pyrrole-2,5-dione derivatives as cholesterol absorption inhibitors for suppressing the formation of foam cells and inflammatory response. *Bioorg Med Chem*. 2018;26:1435–47.
- Palanki MSS, et al. Development of a long acting human growth hormone analog suitable for once a week dosing. *Bioorg Med Chem Lett*. 2013;23:402–6.
- Jones MW, et al. Polymeric dibromomaleimides as extremely efficient disulfide bridging bioconjugation and pegylation agents. *J Am Chem Soc*. 2012;134:1847–52.
- Robin MP, et al. Conjugation-induced fluorescent labeling of proteins and polymers using dithiomaleimides. *J Am Chem Soc*. 2013;135:2875–8.
- Schumacher FF, et al. Next generation maleimides enable the controlled assembly of antibody–drug conjugates via native disulfide bond bridging. *Org Biomol Chem*. 2014;12:7261–9.
- Magdolna Csávás, et al. Synthesis and antibacterial evaluation of some teicoplanin pseudoaglycon derivatives containing alkyl- and arylthiosubstituted maleimides. *J Antibiot*. 2015;68:579–85.
- Zs Szűcs, et al. Lipophilic teicoplanin pseudoaglycon derivatives are active against vancomycin- and teicoplanin-resistant enterococci. *J Antibiot*. 2017;70:664–70.
- Igarashi Y, Watanabe S. Antimicrobial activities of 2-arylthio-N-alkylmaleimides. *J Ind Microbiol*. 1992;9:91–6.
- NCCLS M100–S10 Reference Method for Broth Dilution Antibacterial Susceptibility Testing. National Committee for Clinical Laboratory Standards; 2000.
- CLSI M38-A2. Ed. 2. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. Pennsylvania: Clinical and Laboratory Standards Institute; 2008.
- CLSI M27-S3. Reference method for broth dilution antifungal susceptibility testing of yeasts. Pennsylvania: Clinical and Laboratory Standards Institute; 2013.