# Synthesis of Isomeric, Oxathiolone Fused Chalcones, and Comparison of Their Activity toward Various Microorganisms and Human Cancer Cells Line

Marek Tadeusz Konieczny,*[,a] Wojciech Konieczny,[a] Michał Sabisz,[b] Andrzej Skladanowski,[b] Roland Wakieć,[b] Ewa Augustynowicz-Kopeć,[c] and Zofia Zwolska[c]

[a] *Department of Organic Chemistry, Medical University of Gdańsk; 80–416 Gdańsk, Poland:* [b] *Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology; 80–952 Gdańsk, Poland: and* [c] *Department of Microbiology, National Research Institute of Tuberculosis and Lung Diseases; 01–138 Warsaw, Poland.*

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**Isomeric oxathiolone fused chalcones were prepared by condensation of appropriate acetylbenzo[1,3]-oxathiol-2-ones with benzaldehydes under acidic conditions. The synthesized compounds were screened for cytotoxic activity using HeLa cells, as well as for antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Proteus vulgaris*, antifungal activity against *Candida albicans*, and tuberculostatic activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* strains.**

**Key words** chalcone; oxathiolone; cytostatic; antibacterial; antifungal; tuberculostatic

Chalcones constitute an attractive molecular scaffold in quest for new biologically active compounds.[1,2] Similarly as for other flavonoids, most of the active chalcones is hydroxy substituted, and catechol group often exerts a beneficial effect on the activity. Recently, we have described synthesis and biological properties of oxathiolone fused chalcones **1**, wherein the oxathiolone ring can be considered as a protected bioisoster of catechole.[3] Now, we report synthesis and biological activity of isomeric compounds **2** and **3** (Fig. 1).

The key substrate **7** for synthesis of chalcones **2** was prepared from benzoxathiolone **4**[4,5] by acetylation[5] and Fries rearrangement of the acetoxy derivative **5**. The rearrangement resulted in a mixture of two isomers which were separated by crystallization to give the already known compound **6**[4] and the desired derivative **7**, in a ratio 1 : 3, respectively (Chart 1).

The hydroxyacetophenone **7** was metylated and condensed under acidic conditions with benzaldehydes to related chalcones (Chart 2).

The isomeric chalcones **3** were prepared analogously, starting from benzoxathiolone **10**[6,7] *via* acetoxy derivative **11**.[7] In this case, the Fries rearrangement of the acetoxy derivative **11** resulted in large excess of the isomer **12**. The second isomer **13**, could be isolated by crystallization (yield 4%) of the residues from crystallization of **12** (Charts 3, 4).

The obtained compounds are given in the Table 1.

**Biological Activity** The synthesized compounds (**15**—**26**) were screened for cytotoxic activity using HeLa cells, as well as for antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Proteus vulgaris*, antifungal activity against *Candida albicans*, and tuberculostatic activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* strains. The results are given in the Table 1.

All compound for which the cytostatic activity against HeLa cells could be determined, exhibited weak activity, in the range 5—12 $\mu$M. Compounds of general formula **2** did not influence growth of any of the tested microorganism, and in this aspect, they were similar to isomers of formula **1**.[3]

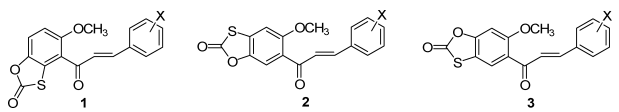

Fig. 1. General Formulas of the Prepared Compounds

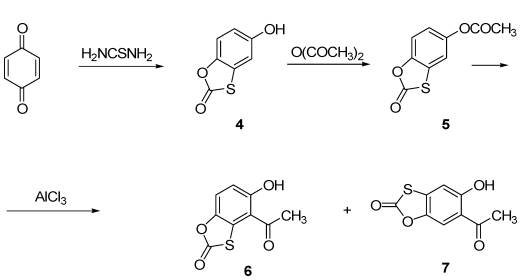

Chart 1. Synthesis of Acetylbenzoxathiolones **6** and **7**

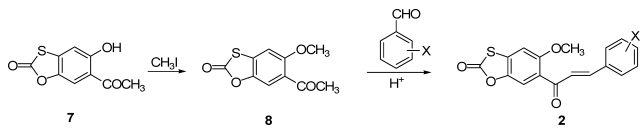

Chart 2. Synthesis of Chalcones of General Structure **2**

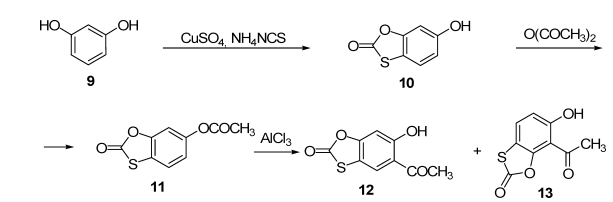

Chart 3. Synthesis of Acetylbenzoxathiolones **12** and **13**

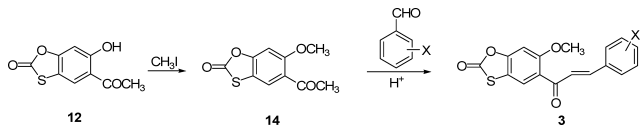

Chart 4. Synthesis of Chalcones of General Structure **3**

* To whom correspondence should be addressed. e-mail: markon@amg.gda.pl

Table 1. Activities of the Synthesized Compounds against Tested Microorganisms and Cell Lines

| Cmpd. | General formula/ substituent | Antifungal activity $IC_{50}$ value ($\mu$g/ml) | Bacteriostatic activity $IC_{50}$ ($\mu$g/ml) | | | | | Cytostatic activity $IC_{50}$ ($\mu$M), [$\mu$g/ml] | Tuberculostatic activity MIC ($\mu$g/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *C. albicans* | *M. luteus* | *St. aureus* | *Sal. typh.* | *E. coli* | *Pr. vulgaris* | HeLa | $H_{37}$Rv | *M. kansasii* |
| **15** | **2**/H | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 7.4 [2.3] | 100 | >100 |
| **16** | **2**/4′-Br | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 11.2 [4.4] | >100 | >100 |
| **17** | **2**/3′-Cl | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 4.9 [1.7] | >100 | >100 |
| **18** | **2**/2′-Cl | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 8.8 [3.0] | >100 | >100 |
| **19** | **2**/4′-$OCH_3$ | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 10.2 [3.5] | >100 | >100 |
| **20** | **2**/3′,4′-di$OCH_3$ | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | —[a] | >100 | >100 |
| **21** | **3**/H | 250 | 62.5 | 31.3 | 125.0 | 125.0 | 125.0 | 8.6 [2.7] | 50 | 100 |
| **22** | **3**/4′-Br | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | 11.4 [4.5] | >100 | >100 |
| **23** | **3**/3′-Cl | 125 | 15.6 | 15.6 | >1000 | >1000 | >1000 | 9.8 [3.4] | 100 | >100 |
| **24** | **3**/2′-Cl | 125 | 31.25 | 15.62 | >1000 | >1000 | >1000 | —[a] | 50 | 100 |
| **25** | **3**/4′-$OCH_3$ | >1000 | 62.5 | 62.5 | >1000 | >1000 | >1000 | —[a] | 50 | >100 |
| **26** | **3**/4′-N$(CH_3)_2$ | >1000 | >1000 | >1000 | >1000 | >1000 | >1000 | —[a] | <25 | 100 |

*a*) The value could not be determined as the compound precipitated out during incubation.

Antimicrobial activity of isomers **3** was strongly influenced by substituents in the ring B (Table 1). The unsubstituted compound of general formula **3** (**21**) was found to be active in all tests, while chloro derivatives **23**, **24** demonstrated antibacterial activity only against *Micrococcus luteus* and *Staphylococcus aureus* strains, and antifungal activity. In contrary, compound **26** was selectively active against *Mycobacterium tuberculosis* $H_{37}$Rv.

## Experimental

**General** Melting points (mp) are uncorrected. Infrared spectra were obtained from KBr pellets on Thermo Mattson Satellite instrument. The $^1$H-NMR spectra were recorded on 200 MHz (Varian Gemini) or 500 MHz (Varian Unity Plus) spectrometers. Elemental analyses were performed on Carlo-Erba 1108 instrument. TLC was carried out on Merck 0.2 mm silica gel 60 F254 aluminum plates.

**Synthesis of 4-Acetyl-5-hydroxybenzo[1,3]oxathiol-2-one (6) and 6-Acetyl-5-hydroxybenzo[1,3]oxathiol-2-one (7)** 5-Acetoxy-benzo[1,3]-oxathiol-2-one (**5**) (21 g, 0.1 mol) and anhydrous aluminum chloride (45 g, 0.33 mol) were carefully mixed and heated at 160 °C for 4 h in a flask equipped in a hydrochloride trap. The hot reaction mixture was treated with water (300 ml) (EXOTHERMIC !), the crude product was filtered off and dried to give mixture of the isomers as a beige solid (19.7 g, 94%). The solid was put in methanol (110 ml), refluxed for 10 min, cooled to room temperature, and the solid was filtered off to give crude isomer **7**. The solid was dissolved in hot toluene (60 ml), filtered while hot, and methanol was added to the filtrate until crystallization started. Filtration gave 6-acetyl-5-hydroxybenzo[1,3]oxathiol-2-one (**7**) as a cream solid (8.8 g, 42%), mp 150—154 °C. *Anal.* Calcd for $C_9H_6O_4S$: C, 51.42; H, 2.88; S, 15.25. Found: C, 51.38; H, 2.80; S, 15.34. IR (KBr) cm$^{-1}$: 1764, 1643, 1475, 1215, 1191. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.64 (s, 3H, $COCH_3$), 7.40 (s, 1H, H-4), 7.94 (s, 1H, H-7), 12.01 (s, 1H, OH). The methanolic filtrates were decolorized with charcoal, evaporated to dryness, and the residue was dissolved in hot toluene (60 ml), filtered while hot, and left for crystallization to give 4-acetyl-5-hydroxybenzo[1,3]oxathiol-2-one (**6**) as yellow solid (3.4 g, 16%). The product was identified by comparison of IR with original sample.[4)]

**Synthesis of 5-Acetyl-6-hydroxybenzo[1,3]oxathiol-2-one (12) and 7-Acetyl-6-hydroxybenzo[1,3]oxathiol-2-one (13)** 6-Acetoxybenzo[1,3]-oxathiol-2-one **11**) (84 g, 0.4 mol) and anhydrous aluminum chloride (240 g, 1.8 mol) were carefully mixed and heated at 160 °C for 4 h in a flask equipped in a hydrochloride trap. The hot reaction mixture was treated with water (EXOTHERMIC !), the crude product was filtered off and dried. The crude product was crystallized from toluene to give 5-acetyl-6-hydroxybenzo[1,3]oxathiol-2-one (**12**) as a colorless solid (40 g, 48%), mp 119—121 °C. *Anal.* Calcd for $C_9H_6O_4S$: C, 51.42; H, 2.88; S, 15.25. Found: C, 51.36; H, 2.90; S, 15.10. IR (KBr) cm$^{-1}$: 1753, 1640, 1613, 1468, 1250, 1168. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.61 (s, 3H, $COCH_3$), 7.10 (s, 1H, H-7), 8.32 (s, 1H, H-4), 12.33 (s, 1H, OH). The filtrates were evaporated to drynnes and crystallized four times from methanol to give 7-acetyl-6-hydroxybenzo[1,3]oxathiol-2-one (**13**) as an orange solid (3.36 g, 4%), mp 137—140 °C. *Anal.* Calcd for $C_9H_6O_4S$: C, 51.42; H, 2.88; S, 15.25. Found: C, 51.35; H, 2.70; S, 15.07. IR (KBr) cm$^{-1}$: 1760, 1640, 1571, 1463, 1211, 1017. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.64 (s, 3H, $COCH_3$), 6.93 (d, 1H, $J$=8.8 Hz, H-5), 7.76 (d, 1H, $J$=8.8 Hz, H-4), 11.75 (s, 1H, OH).

**Synthesis of 6-Acetyl-5-methoxybenzo[1,3]oxathiol-2-one (8)** 6-Acetyl-5-hydroxybenzo[1,3]oxathiol-2-one (**7**) (8.4 g, 40 mmol), anhydrous potassium carbonate (16.58 g, 120 mmol) and methyl iodide (7.5 ml, 120 mmol) in dry DMF (75 ml) were stirred at room temperature for 3 h. The reaction mixture was diluted with water, the precipitated solid was filtered off, dried and crystallized from methanol to give 6-acetyl-5-methoxybenzo[1,3]oxathiol-2-one (**8**) (4.43 g, 50%) as a colorless solid, mp 138—140 °C. *Anal.* Calcd for $C_{10}H_8O_4S$: C, 53.56; H, 3.60; S, 14.30. Found: C, 53.37; H, 3.70; S, 14.15. IR (KBr) cm$^{-1}$: 1760, 1654, 1408, 1266, 1018. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.57 (s, 3H, $COCH_3$), 3.92 (s, 3H, $OCH_3$), 7.62 (s, 1H, H-4), 7.72 (s, 1H, H-7).

**Synthesis of 5-Acetyl-6-methoxybenzo[1,3]oxathiol-2-one (14)** 5-Acetyl-6-hydroxybenzo[1,3]oxathiol-2-one (**12**) (4.2 g, 20 mmol), anhydrous potassium carbonate (9.68 g, 70 mmol) and methyl iodide (3.7 ml, 60 mmol) in dry DMF (35 ml) were stirred at room temperature for 3 h. The reaction mixture was diluted with water, the precipitated solid was filtered off, dried and crystallized from ethanol to give 5-acetyl-6-methoxybenzo[1,3]oxathiol-2-one (**8**) (2.77 g, 60%) as a colorless solid, mp 144—145 °C. *Anal.* Calcd for $C_{10}H_8O_4S$: C, 53.56; H, 3.60; S, 14.30. Found: C, 53.41; H, 3.55; S, 14.19. IR (KBr) cm$^{-1}$: 1761, 1666, 1605, 1468, 1277, 1005. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.53 (s, 3H, $COCH_3$), 3.93 (s, 3H, $OCH_3$), 7.42 (s, 1H, H-7), 8.02 (s, 1H, H-4).

**General Procedure for Condensation of 6-Acetyl-5-methoxybenzo-[1,3]oxathiol-2-one (8) and 5-Acetyl-6-methoxybenzo[1,3]oxathiol-2-one (14) with Benzaldehydes** Benzo[1,3]oxathiol-2-one derivative (**8** or **14**) (224 mg, 1 mmol), a suitable benzaldehyde (1.5 mmol) and conc. sulfuric acid (0.2 ml) in acetic acid (2 ml) were stirred at 60—80 °C until completion of the reaction (2—3 h). The cooled mixture was usually diluted with methanol or water (1—5 ml) and the precipitated solid was filtered off and washed with methanol. The crude product was often contaminated by a red compound, which was typically removed by dissolving the product in acetone and filtration by a silica gel pad. The filtrate was evaporated and crystallized.

**5-Methoxy-6-(3-phenylacryolyl)benzo[1,3]oxathiol-2-one (15)** Reaction of **8** with benzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with water, filtration through silica gel pad and crystallization from 2-methoxyethanol. Rosy solid, yield 42%, mp 168—170 °C. *Anal.* Calcd for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87; S, 10.27. Found: C, 65.20; H, 3.79; S, 10.14. IR (KBr) cm$^{-1}$: 1758, 1629, 1411, 1023. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.87 (s, 3H, $OCH_3$), 7.35—7.53 (m, 5H, H-$\alpha$, H-$\beta$, H-3′, H-4′, H-5′), 7.60 (s, 1H, H-4), 7.75 (m, 2H, H-2′, H-6′), 7.71 (s, 1H, H-7).

**6-[3-(4′-Bromophenyl)acryolyl]-5-methoxybenzo[1,3]oxathiol-2-on (6)** Reaction of **8** with 4-bromobenzaldehyde, reaction temperature 70 °C; time

2.5 h. The product was isolated by precipitation with methanol, filtration through silica gel pad and crystallization from 2-methoxyethanol. Colorless solid, yield 30%, mp 189—190 °C. *Anal.* Calcd for $C_{17}H_{11}BrO_4S$: C, 52.19; H, 2.83; S, 8.20. Found: C, 52.01; H, 2.90; S, 8.07. IR (KBr) $cm^{-1}$: 1760, 1629, 1414, 1020. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 3.87 (s, 3H, $OCH_3$), 7.72 (d, 2H, $J$=8.3 Hz, H-2′, H-6′), 7.44 (d, 1H, $J$=16.1 Hz, H-$\alpha$), 7.52 (d, 1H, $J$=16.1 Hz, H-$\beta$), 7.60 (s, 1H, H-4), 7.64 (d, 2H, $J$=8.3 Hz, H-3′, H-5′), 7.71 (s, 1H, H-7).

**6-[3-(3′-Chlorophenyl)acryolyl]-5-methoxybenzo[1,3]oxathiol-2-one (17)** Reaction of **8** with 3-chlorobenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with water, washing with methanol and crystallization from 2-methoxyethanol methanol mixture. Salmon-pink solid, yield 35%, mp 122—124 °C. *Anal.* Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.98; H, 3.15; S, 9.07. IR (KBr) $cm^{-1}$: 1754, 1655, 1589, 1411, 1023. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.89 (s, 3H, $OCH_3$), 7.4—8.0 (m, 8H, aromatic+olefinic).

**6-[3-(2′-Chlorophenyl)acryolyl]-5-methoxybenzo[1,3]oxathiol-2-one (18)** Reaction of **8** with 2-chlorobenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with water, washing with methanol, filtration through silica gel pad and crystallization from acetone. Cream solid, yield 42%, mp 165—168 °C. *Anal.* Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.75; H, 3.31; S, 9.08. IR (KBr) $cm^{-1}$: 1786, 1648, 1578, 1413, 1270, 1014. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 3.89 (s, 3H, $OCH_3$), 7.40—7.54 (m, 3H, H-$\alpha$, H-4′, H-5′), 7.57 (d, 1H, $J$=7.8 Hz, H-3′), 7.73 (s, 1H, H-7), 7.64 (s, 1H, H-4), 7.84 (d, 1H, $J$=16.1 Hz, H-$\beta$), 7.98 (d, 1H, $J$=7.3 Hz, H-6′).

**6-[3-(4′-Methoxyphenyl)acryolyl]-5-methoxybenzo[1,3]oxathiol-2-one (19)** Reaction of **8** with 4-methoxybenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with water, washing with methanol, filtration through silica gel pad and crystallization from 2-methoxyethanol. Yellow solid, yield 40%, mp 190—193 °C. *Anal.* Calcd for $C_{18}H_{14}O_5S$: C, 63.15; H, 4.12; S, 9.37. Found: C, 63.32; H, 4.03; S, 9.26. IR (KBr) $cm^{-1}$: 1742, 1600, 1242, 1024. $^1$H-NMR (500 MHz, acetone-$d_6$) $\delta$: 3.88 (s, 3H, $OCH_3$), 4.00 (s, 3H, $OCH_3$), 7.02 (d, 2H, $J$=8.8 Hz, H-3′, H-5′), 7.37 (d, 1H, $J$=15.6 Hz, H-$\alpha$), 7.61 (s, 1H, H-7), 7.55 (s, 1H, H-4), 7.62 (d, 1H, $J$=15.6 Hz, H-$\beta$), 7.71 (d, 2H, $J$=8.8 Hz, H-2′, H-6′).

**6-[3-(3′,4′-Dimethoxyphenyl)acryolyl]-5-methoxybenzo[1,3]oxathiol-2-one (20)** Reaction of **8** with 3,4-dimethoxybenzaldehyde, reaction temperature 70 °C; time 2.5 h. The reaction mixture was diluted with ethyl acetate, washed with water, dried (sodium sulfate), and evaporated. The residue was purified on silica gel column in chloroform. The main fraction was crystallized from 2-methoxyethanol to give cream solid, yield 25%, mp 183—186 °C. *Anal.* Calcd for $C_{19}H_{16}O_6S$: C, 61.28; H, 4.33; S, 8.61. Found: C, 61.05; H, 4.24; S, 8.47. IR (KBr) $cm^{-1}$: 1738, 1621, 1591, 1203, 1019. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.81 (s, 6H, 2×$OCH_3$), 3.85 (s, 3H, $OCH_3$), 7.00 (d, 1H, $J$=8.3 Hz, H-5′), 7.20—7.38 (m, 3H, H-$\alpha$, H-2′, H-6′), 7.45 (d, 1H, $J$=16 Hz, H-$\beta$), 7.56 (s, 1H, H-4), 7.70 (s, 1H, H-7).

**6-Methoxy-5-(3-phenylacryolyl)benzo[1,3]oxathiol-2-one (21)** Reaction of **14** with benzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with methanol, and crystallization from 2-methoxyethanol. Rosy solid, yield 32%, mp 171—175 °C. *Anal.* Calcd for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87; S, 10.27. Found: C, 65.30; H, 3.78; S, 10.07. IR (KBr) $cm^{-1}$: 1754, 1609, 1571, 1266. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 3.91 (s, 3H, $OCH_3$), 7.38—7.48 (m, 5H, H-$\alpha$, H-7, H-3′, H-4′, H-5′), 7.56 (d, 1H, $J$=16.2 Hz, H-$\beta$), 7.74 (bs, 2H, H-2′, H-6′), 7.95 (s, 1H, H-4).

**5-[3-(4′-Bromophenyl)acryolyl]-6-methoxybenzo[1,3]oxathiol-2-one (22)** Reaction of **14** with 4-bromobenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with methanol, and crystallization from 2-methoxyethanol. Orange solid, yield 78%, mp 216—220 °C. *Anal.* Calcd for $C_{17}H_{11}BrO_4S$: C, 52.19; H, 2.83; S, 8.20. Found: C, 52.12; H, 2.74; S, 8.25. IR (KBr) $cm^{-1}$: 1763, 1612, 1412, 1026. $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta$: 3.91 (s, 3H, $OCH_3$), 7.44 (d, 1H, $J$=16.1 Hz, H-$\alpha$), 7.47 (s, 1H, H-7), 7.53 (d, 1H, $J$=16.1 Hz, H-$\beta$), 7.64 (d, 2H, $J$=8.3 Hz, H-3′, H-5′), 7.71 (d, 2H, $J$=8.3 Hz, H-2′, H-6′), 7.95 (s, 1H, H-4).

**5-[3-(3′-Chlorophenyl)acryolyl]-6-methoxybenzo[1,3]oxathiol-2-one (23)** Reaction of **14** with 3-chlorobenzaldehyde, reaction temperature 70 °C; time 2 h. The product was isolated by precipitation with methanol, filtration, washing with methanol and crystallization from 2-methoxyethanol. Salmon-pink solid, yield 50%, mp 183—186 °C. *Anal.* Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.76; H, 3.09; S, 9.11. IR (KBr) $cm^{-1}$: 1762, 1611, 1470, 1278, 1198, 1012. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.91 (s, 3H, $OCH_3$), 7.4—8.0 (m, 8H, aromatic+olefinic).

**5-[3-(2′-Chlorophenyl)acryolyl]-6-methoxybenzo[1,3]oxathiol-2-one (24)** Reaction of **14** with 2-chlorobenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by filtration, washing with methanol, reflux in 2-methoxyethanol, filtration and washing with methanol. Cream solid, yield 60%, mp 245—250 °C. *Anal.* Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.70; H, 3.12; S, 9.09. IR (KBr) $cm^{-1}$: 1753, 1608, 1463, 1270, 1178, 1002. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.93 (s, 3H, $OCH_3$), 7.38—7.62 (m, 5H, H-$\alpha$, H-7, H-3′, H-4′, H-5′), 7.85 (d, 1H, $J$=15.9 Hz, H-$\beta$), 8.00 (s, 1H, H-4), 7.98 (m, 1H, H-6′).

**5-[3-(4′-Methoxyphenyl)acryolyl]-6-methoxybenzo[1,3]oxathiol-2-one (25)** Reaction of **14** with 4-methoxybenzaldehyde, reaction temperature 70 °C; time 2.5 h. The product was isolated by precipitation with methanol, filtration and washing with methanol. The crude product was purified on short silica gel column in chloroform–ethyl acetate 3 : 1 solution, and crystallized from 2-methoxyethanol. Beige solid, yield 32%, mp 202—206 °C. *Anal.* Calcd for $C_{18}H_{14}O_5S$: C, 63.15; H, 4.12; S, 9.37. Found: C, 63.10; H, 4.03; S, 9.21. IR (KBr) $cm^{-1}$: 1748, 1603, 1510, 1267, 1171, 1018. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.81 (s, 3H, $OCH_3$), 3.90 (s, 3H, $OCH_3$), 7.00 (d, 2H, $J$=8.8 Hz, H-3′, H-5′), 7.26 (d, 1H, $J$=15.8 Hz, H-$\alpha$), 7.52 (d, 1H, $J$=15.8 Hz, H-$\beta$), 7.45 (s, 1H, H-7), 7.91 (s, 1H, H-4), 7.71 (d, 2H, $J$=8.8 Hz, H-2′, H-6′).

**5-[3-(4′-Dimethylaminophenyl)acryolyl]-6-methoxybenzo[1,3]-oxathiol-2-one (26)** Reaction of **14** with 4-dimethylaminobenzaldehyde, reaction temperature 70 °C; time 3 h. The product was isolated by precipitation with methanol and filtration. The crude product was purified on short silica gel column in chloroform, and crystallized from 2-methoxyethanol. Yellow solid, yield 40%, mp 209—213 °C. *Anal.* Calcd for $C_{19}H_{17}NO_4S$: C, 64.21; H, 4.82; N, 3.94; S, 9.02. Found: C, 64.13; H, 4.73; N, 3.77; S, 9.11. IR (KBr) $cm^{-1}$: 1758, 1604, 1526, 1171, 1011. $^1$H-NMR (200 MHz, DMSO-$d_6$) $\delta$: 3.00 (s, 6H, $N(CH_3)_2$), 3.88 (s, 3H, $OCH_3$), 6.72 (d, 2H, $J$=8.8 Hz, H-3′, H-5′), 7.09 (d, 1H, $J$=15.8 Hz, H-$\alpha$), 7.43 (d, 1H, $J$=15.8 Hz, H-$\beta$), 7.55 (d, 2H, $J$=8.8 Hz, H-2′, H-6′), 7.43 (s, 1H, H-7), 7.86 (s, 1H, H-4).

**Cytotoxicity Assays** Cell lines: Human cervix carcinoma HeLa S3 cells were maintained in high glucose DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine and antibiotics (100 units/ml penicillin and 100 $\mu$g/ml streptomycin) at 37 °C in 10% $CO_2$/air atmosphere. Cells were screened routinely for Mycoplasma by the PCR method with Mycoplasma *Plus* PCR Primer Set (Stratagene, La Jolla, CA, U.S.A.).

The cytotoxicity was determined by the MTT assay. Briefly, exponentially growing cells were attached at $3\times10^4$ cells/well in a 24-well multiwell plates and the cellular viability was determined after 120 h of continuous exposure to different drug concentrations. Cells were incubated with the MTT tetrazolium salt for 4 h at 37 °C, and the formation of formazan was measured by a microplate reader. The concentrations required to inhibit cell growth by 50% compared to untreated controls were determined from the curves plotting survival as a function of dose by use of the Slide Write program. All values are averages of at least two independent experiments, each done in duplicate.

**Determination of Tuberculostatic Activity** Tuberculostatic activity was tested with the test tube method of Youman's liquid medium containing 10% of bovine serum, toward *Mycobacterium tuberculosis* $H_{37}$Rv and *Mycobacterium kansasii* strains with rifampicine (RMP) as a drug control. The minimum inhibiting concentration (MIC) for RMP was 6.2 $\mu$g/ml both for *M.* $H_{37}$Rv and *M. kansasii*.

**Determination of Antibacterial and Antifungal Activity** Antibacterial and antifungal activity was determined by the serial twofold dilution microtiter plate method, in Nutrient Broth medium (Becton Dickinson/Difco), for antibacterial activity determination or in YEPG medium (1% Yeast extract, 1% Bacto-Peptone, 2% glucose) for antifungal activity determination. Wells containing serially diluted examined compounds and inhibitor-free control were inoculated with overnight cultures of tested cells to the final concentration of $10^4$ cells/ml. Plates were then incubated for 24 h at 37 °C (antibacterial activity determination) or for 48 h at 30 °C (antifungal activity determination). Microbial growth was quantified in each well by the measurement of an optical density at $\lambda$=595 nm using the microplate reader (Labsystems, Multiscan Bichromatic). Drug concentrations causing 50% reduction of microbial growth in comparison to the drug-free control ($IC_{50}$) was read from the $A_{595}=f(\log c)$ curves, where $A_{595}$ is the absorbance at $\lambda$=595 nm and $c$ - concentration of a tested compound in $\mu$g/ml. The following microbial strains were used: a/bacteria - *Micrococcus luteus* (clinical isolate from the collection of Medical University of Gdańsk), *Staphylococcus aureus* ATCC 9144, *Salmonella typhimurium* PCM 2180, *Escherichia coli* ATCC 11775, *Proteus vulgaris* ATCC 6380; b/fungi - *Candida albicans* ATCC 10261.

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