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Research Article

Influence of S-Oxidation on Cytotoxic Activity of Oxathiole-Fused Chalcones

Marek T. Konieczny[1,*], Anita Bułakowska[2], Danuta Pirska[2], Wojciech Konieczny[2], Andrzej Skladanowski[3], Michal Sabisz[3,†], Marek Wojciechowski[3] and Krzysztof Lemke[4,‡]

[1]*Department of Chemical Technology of Drugs, Medical University of Gdansk, Gen. J. Hallera Street 107, 80-416 Gdansk, Poland*
[2]*Department of Organic Chemistry, Medical University of Gdansk, 80-416 Gdansk, Poland*
[3]*Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, 80-233 Gdansk, Poland*
[4]*Drug Discovery Department, Adamed Group, 05-152 Czosnow, Poland*
**Corresponding author: Marek T. Konieczny, markon@gumed.edu.pl*
[†]Present Addresses: Laboratory of Therapeutic Proteins and Peptides, Ecole Polytechnique Federale de Lausanne, CH – 1015, Lausanne, Switzerland
[‡]Research and Development Department Biovico Ltd. 81-340 Gdynia Poland

Synthesis, *in vitro* cytotoxic activity, and interaction with tubulin of oxidized, isomeric 1-(5-alkoxybenzo[*d*][1,3]oxathiol-6-yl)-3-phenylprop-2-en-1-ones and 1-(6-alkoxybenzo[*d*][1,3]oxathiol-5-yl)-3-phenylprop-2-en-1-ones are described. Most of the compounds demonstrated cytotoxic activity at submicromolar concentrations. It was found that oxidation of sulfur atom of the oxathiole-fused chalcones strongly influenced activity of the parent compounds, and that depending on relative position of the sulfur atom in the molecule, the activity was either increased or diminished. For isomers with sulfur atom *para* to the chalcone carbonyl group, oxidation led to increase in activity, while for isomers with sulfur atom *meta* to the carbonyl the activity dropped down. It was demonstrated that the compounds interact with tubulin at the colchicine binding site, and the interaction was evaluated using molecular modeling. It was concluded that the observed profound influence of oxidation of the sulfur atom on cytotoxic activity cannot be solely related to interaction of the compounds with tubulin.

Key words: benzoxathiole oxidation, chalcone, cytotoxic activity, molecular modeling, tubulin interaction

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Chalcones are widely explored as potential lead compounds for new therapeutic agents, and antitumor properties of the compounds are among the most often studied (1–17).

We have already reported synthesis and cytotoxic activity of isomeric oxathiole-fused chalcones **1** (18) and **2** (19), and their dioxole analogs **3** (18) (Figure 1). It was found that cytotoxic activity of the compounds depended strongly on the substituents OR, X, and on the presence of heterocyclic ring, but was almost insensitive to position of the sulfur atom, or even its replacement by oxygen (18,19).

Promising activity of compounds **1** and **2** prompted us to synthesize their analogs with the sulfur atom oxidized either to sulfoxide (**4**, **5**) or sulfone (**6**, **7**) (Figure 1). It was speculated that such modification could increase metabolic stability of the compounds, as oxidation of sulfur atom to sulfoxide or sulfone constitutes a dominating metabolic transformation of divalent sulfur compounds (20–22). Generally, influence of oxidation of sulfur compounds on their biological activity seems to be unpredictable, as nicely summarized by Prof. A. G. Renwick in his review on pharmacological and toxicological consequences of sulphoxide oxidation and reduction: "Thus overall conclusions and generalizations concerning the effects of changes in redox state on pharmacological or toxicological activity are probably best avoided" (21,22). The obtained compounds were tested for their cytotoxic activity *in vitro*, and mechanism of the activity was probed using assays for their interaction with tubulin, and molecular modeling.

Experimental

Chemistry

General

Melting points were determined using a Stuart SMP3 instrument and were uncorrected. Infrared spectra were obtained from KBr pellets on a Thermo Mattson Satellite instrument. The ^{1}H NMR spectra were recorded on

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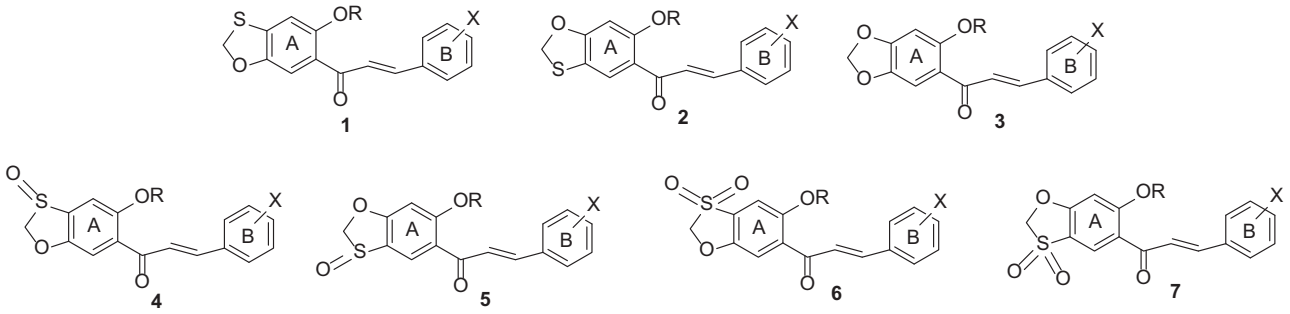


Figure 1: Structures of the studied compounds (**4**–**7**) and their precursors (**1**–**3**).

200 MHz (Varian Gemini) or 500 MHz (Varian Unity Plus) spectrometers and the chemical shifts were expressed in δ (ppm) values (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Elemental analyses were performed using a Carlo-Erba 1108 instrument and results were within 0.4% of theoretical values. The described reactions are unoptimized.

General procedure for oxidation of chalcones to their S-monooxido derivatives

A suitable chalcone (1 mmol) was dissolved in acetic acid (15 mL), 30% hydrogen peroxide (3 mL) was added and the mixture was stirred at room temperature for about 2 h. Icy water was added to the obtained solution and the precipitated solid was filtered off, washed with water, dried and crystallized from a suitable solvent, usually methanol or ethanol.

(*E*)-1-(5-Methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-phenylprop-2-en-1-one (**4a**), crystallization solvent: methanol, a colorless solid, yield 82%, mp 220–222 °C.

IR (KBr, cm^{-1}): 3059, 1633, 1475, 1053.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.95 (s, 1H, H-4), 7.75 (m, 2H, H-2′, H-6′), 7.42–7.50 (m, 4H, H-3′, H-4′, H-5′, H-β), 7.38 (s, 1H, H-7), 7.30 (d, 1H, J = 16.1 Hz, H-α), 5.60 (d, 1H, J = 11.2 Hz, H-2), 5.20 (d, 1H, J = 11.2 Hz, H-2), 3.87 (s, 3H, OCH_3).

EA: Calcd for $C_{17}H_{14}O_4S$: C, 64.95; H, 4.49; S, 10.20. Found: C, 65.33; H, 4.45; S, 10.33.

(*E*)-3-(3-Chlorophenyl)-1-(5-methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**4b**), crystallization solvent: methanol, a cream solid, yield 80%, mp 185–186 °C.

IR (KBr, cm^{-1}): 3065, 1654, 1586, 1471, 1010.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.96 (s, 1H, H-4), 7.90 (s, 1H, H-2′), 7.74 (d, 1H, J = 7.3 Hz, H-6′), 7.44–7.54 (m, 3H, H-4′, H-5′, H-β), 7.39 (s, 1H, H-7), 7.38 (d, 1H, J = 16.1 Hz, H-α), 5.65 (d, 1H, J = 11.2 Hz, H-2), 5.19 (d, 1H, J = 11.2 Hz, H-2), 3.86 (s, 3H, OCH_3).

EA: Calcd for $C_{17}H_{13}ClO_4S$:, 58.54; H, 3.76; S, 9.19. Found: 58.31; H, 3.66; S, 8.99.

(*E*)-1-(5-Methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**4c**), crystallization solvent: methanol, a cream solid, yield 85%, mp 201–202 °C.

IR (KBr, cm^{-1}): 1634, 1602, 1512, 1401, 1255, 1171.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.93 (s, 1H, H-4), 7.71 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.41 (d, 1H, J = 16.1 Hz, H-β), 7.35 (s, 1H, H-7), 7.14 (d, 1H, J = 16.1 Hz, H-α), 6.99 (d, 2H, J = 8.3 Hz, H-3′, H-5′), 5.64 (d, 1H, J = 11.7 Hz, H-2), 5.19 (d, 1H, J = 11.7 Hz, H-2), 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.93, 162.26, 153.79, 153.06, 145.73, 136.21, 133.10, 133.07, 131.43, 127.48, 124.90, 115.21, 114.17, 113.25, 111.26, 92.03, 57.43, 56.08.

EA: Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68; S, 9.31. Found: C, 62.61; H, 4.65; S, 9.33.

(*E*)-3-(3-Hydroxy-4-methoxyphenyl)-1-(5-methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**4d**), crystallization solvent: ethanol, a yellow solid, yield 74%, mp 184–186 °C.

IR (KBr, cm^{-1}): 3240, 2984, 1653, 1569, 1510, 1400, 1254, 1010, 807.

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.26 (s, 1H, OH), 7.94 (s, 1H, H-4), 7.33 (m, 2H, H-2′, H-β), 7.16 (m, 2H, H-6′, H-7), 7.02 (d, 1H, J = 16.1 Hz, H-α), 6.97 (d, 1H, J = 8.3 Hz, H-5′), 5.65 (d, 2H, J = 11.2 Hz, H-2), 5.20 (d, 2H, J = 11.2 Hz, H-2), 3.86 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.67, 153.80, 153.09, 151.28, 147.43, 146.12, 136.17, 133.13, 127.71, 124.64,

122.96, 114.95, 113.30, 112.66, 111.29, 92.03, 57.44, 56.34.

EA: Calcd for $C_{18}H_{16}O_6S$: C, 59.99; H, 4.47; S, 8.90. Found: C, 59.85;H, 4.38; S, 9.05.

(*E*)-3-(3-Fluoro-4-methoxyphenyl)-1-(5-methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**4e**), crystallization solvent: ethanol, a colorless solid, yield 80%, mp 204–207 °C.

IR (KBr, cm^{-1}): 2949, 1605, 1521, 1476, 1403, 1289, 1040, 819.

^1H NMR (500 MHz, DMSO-d_6): δ 7.95 (s, 1H, H-4), 7.76 (dd, 1H, J_1 = 1.9 Hz, J_2 = 12.7 Hz H-2′), 7.56 (d, 1H, J = 8.3 Hz, H-6′), 7.40 (d, 1H, J = 16.1 Hz, H-β,), 7.36 (s, 1H, H-7), 7.22 (m, 2H, H-5′, H-α), 5.65 (d, 1H, J = 11.2 Hz, H-2), 5.20 (d, 1H, J = 11.2 Hz, H-2), 3.89 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.94, 153.77, 153.18, 153.11, 151.23, 150.12, 150.04, 144.59, 136.02, 133.19, 128.14, 128.09, 127.61, 127.59, 126.22, 115.96, 115.81, 114.52, 113.28, 111.30, 92.04, 57.44, 56.86.

EA: Calcd for $C_{18}H_{15}FO_5S$: C, 59.66; H, 4.17; S, 8.85. Found: C, 59.52; H, 4.16; S, 8.84.

(*E*)-1-(5-Methoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**4f**), purified on silica gel column in methylene chloride, followed by crystallization from ethanol, a yellow solid, yield 50%, mp 209–210 °C.

IR (KBr, cm^{-1}): 2944, 1655, 1603, 1561, 1470, 1337, 1203, 1037, 1037, 1009.

^1H NMR (500 MHz, DMSO-d_6): δ 7.92 (s, 1H, H-4), 7.83 (d, 1H, J = 16.1 Hz, H-β), 7.40 (d, 1H, J = 16.1 Hz, H-α), 7.31 (s, 1H, H-7), 6.30 (s, 2H, H-3′, H-4′), 5.64 (d, 1H, J = 11.2 Hz H-2), 5.20 (d, 1H, J = 11.2 Hz, H-2,), 3.86 (s, 12H, 4 × OCH_3).

EA: Calcd for $C_{20}H_{20}O_7S$: C, 59.40; H, 4.98; S, 7.93. Found: C, 58.97; H, 5.00; S, 7.80.

(*E*)-3-(3-Hydroxy-4-methoxyphenyl)-1-(3-oxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**4g**), purified on silica gel column in methylene chloride, followed by crystallization from ethanol, an orange solid, yield 41%, mp 144–145 °C.

IR (KBr): 3230, 1641, 1555, 1523, 1440, 1269, 1134, 1011.

^1H NMR (500 MHz, DMSO-d_6): δ 9.26 (s, 1H, OH), 7.92 (s, 1H, H-4), 7.36 (m, 2H, H-7, H-β), 7.15 (m, 2H, H-2′, H-6′), 7.08 (d, 1H, J = 15.9 Hz, H-α), 6.97 (d, 1H, J = 7.8 Hz, H-5′), 5.64 (d, 1H, J = 11.3 Hz, H-2), 5.19 (d, 1H, J = 11.3 Hz, H-2), 4.06 (s, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 1.68 (m, 2H, CH_2), 0.89 (t, 3H, J = 6.9 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.50, 153.71, 152.57, 151.20, 147.41, 145.53, 136.29, 133.37, 127.78, 124.63, 122.79, 114.90, 113.40, 112.65, 112.05, 92.00, 71.31, 56.32, 22.63, 11.12.

EA: Calcd for $C_{20}H_{20}O_6S$: C, 61.84; H, 5.19; S, 8.25. Found: C, 61.69; H, 5.40; S, 8.04.

Sodium (*E*)-2-methoxy-5-(3-(3-oxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-oxoprop-1-en-1-yl)phenyl phosphate (**4h**). The starting (*E*)-2-methoxy-5-(3-oxo-3-(5-propoxybenzo[*d*][1,3]oxathiol-6-yl)prop-1-en-1-yl)phenyl dihydrogen phosphate (1.274 g, 2.81 mmol) was stirred in acetic acid (25 mL) and hydrogen peroxide (9 mL) for 4 h. The obtained yellow solid was filtered off, and washed with acetic acid and next with ethyl ether. The obtained solid was suspended in cold methanol (5 mL) and 1 N solution of sodium hydroxide in methanol (5 mL) was added, to give an orange solution. The solution was concentrated on rotary evaporator, and ethanol (3 mL) was added to the residue. The precipitate was filtered off and washed with ethanol to give a beige solid, yield 1.0 g, 70%, mp 205–208 °C.

IR (KBr, cm^{-1}): 3411, 1655, 1595, 1509, 1270, 1134, 985.

^1H NMR (500 MHz, D_2O): δ 7.64 (s, 1H, H-4), 7.61 (d, 1H, J = 2.0 Hz, H-2′), 7.33 (d, 1H, J = 16.0 Hz, H-β), 7.23 (s, 1H, H-7), 7.17 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.0 Hz, H-6′), 6.98 (d, 1H, J = 16.0 Hz, H-α), 6.93 (d, 1H, J = 8.5 Hz, H-5′), 5.58 (d, 1H, J = 11.8 Hz, H-2), 5.15 (d, 1H, J = 11.8 Hz, H-2), 3.94 (t, 2H, J = 6.4 Hz, OCH_2), 3.77 (s, 3H, OCH_3), 1.56 (m, 2H, CH_2), 0.75 (t, 3H, J = 7.4 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.58, 153.71, 152.35, 147.85, 136.79, 132.96, 128.00, 122.97, 115.18, 113.19, 111.93, 111.61, 91.97, 71.25, 55.78, 22.59, 11.07.

EA: Calcd for $C_{20}H_{19}Na_2O_9PS$ × 3 H_2O: C, 42.41; H, 4.45; S, 5.66. Found: C, 42.67; H, 4.31; S, 5.69.

(*E*)-1-(3-Oxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**4i**), crystallization solvent: methanol, a yellow solid, yield 45%, mp 183–184 °C.

IR (KBr): 1651, 1607, 1563, 1460, 1329, 1206, 1124, 1060, 1006.

^1H NMR (500 MHz, DMSO-d_6): δ 7.90 (S, 1H, H-4), 7.86 (d, 1H, J = 16.1 Hz, H-β), 7.41 (d, 1H, J = 16.1 Hz, H-α), 7.28 (s, 1H, H-7), 6.30 (s, 2H, H-3′, H-5′), 5.63 (d, 1H, J = 11.4 Hz, H-2), 5.19 (d, 1H, J = 11.4 Hz, H-2), 4.06 (t,

2H, J = 6.3 Hz, OCH_2), 3.86 (s, 9H, 3 × OCH_3), 1.68 (m, 2H, CH_2), 0.90 (t, 3H, J = 7.4 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 193.69, 164.23, 162.04, 153.75, 152.41, 137.33, 136.08, 133.04, 125.91, 113.27, 112.00, 105.35, 91.97, 91.72, 71.33, 56.72, 56.28, 22.56, 10.95.

EA: Calcd for $C_{22}H_{24}O_7S$: C, 61.10; H, 5.59; S, 7.41. Found: C, 60.86; H, 5.58; S, 7.45.

(*E*)-3-(3-Fluoro-4-methoxyphenyl)-1-(3-oxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**4j**), crystallization solvent: ethanol, a cream solid, yield 84%, mp 158–160 °C.

IR (KBr, cm^{-1}): 1662, 1608, 1592, 1516, 1283, 1130, 1041.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.93 (s, 1H, H-4), 7.74 (dd, 1H, J_1 = 12.7 Hz, J_2 = 2.0 Hz, H-2′), 7.56 (d, 1H, J = 8.3 Hz, H-6′), 7.43 (d, 1H, J = 16.1 Hz, H-β), 7.36 (s, 1H, H-7), 7.26 (d, 1H, J = 16.1 Hz, H-α), 7.22 (t, 1H, J = 8.7 Hz, H-5′), 5.65 (d, 1H, J = 11.2 Hz, H-2), 5.20 (d, 1H, J = 11.2 Hz, H-2),4.06 (t, 2H, J = 6.0 Hz, OCH_2), 3.90 (s, 3H, OCH_3), 1.67 (m, 2H, CH_2), 0.88 (t, 3H, J = 7.3 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.77, 153.69, 153.16, 152.62, 151.22, 150.04, 149.95, 143.92, 136.12, 133.44, 128.21, 128.15, 127.41, 127.39, 126.28, 115.86, 115.71, 114.54, 113.37, 112.05, 92.01, 71.28, 56.84, 22.61, 11.09.

EA: Calcd for $C_{20}H_{19}FO_5S$: C, 61.53; H, 4.91; S, 8.21. Found: C, 61.60; H, 4.86; S. 8.08.

(*E*)-1-(5-Butoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**4k**), crystallization solvent: methanol, a yellow solid, yield 73%, mp 129–130 °C.

IR (KBr): 1655, 1578, 1504, 1464, 1419, 1274, 1128, 1010.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.95 (s, 1H, H-4), 7.42 (d, 1H, J = 16.0 Hz, H-β),7.36 (s, 1H, H-7), 7.34 (d, 1H, J = 16.0 Hz, H-α), 7.11 (s, 2H, H-2′, H-6′), 5.65 (d, 1H, J = 11.4 Hz, H-2), 5.20 (d, 1H, J = 11.4 Hz, H-2), 4.11 (t, 1H, J = 6.3 Hz, OCH_2), 3.82 (s, 6H, 2 × OCH_3), 3.71 (s, 3H, OCH_3), 1.65 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 0.79 (t, 3H, J = 7.4 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 193.00, 153.75, 153.67, 152.66, 145.37, 140.43, 136.19, 133.37, 130.57, 126.81, 113.30, 112.02, 106.89, 92.01, 69.46, 60.81, 56.70, 31.28, 19.39, 14.21.

EA: Calcd for $C_{23}H_{26}O_7S$: C, 61.87; H, 5.87; S, 7.18. Found: C, 61.67; H, 5.81; S, 7.07.

(*E*)-1-(5-Butoxy-3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (**4l**), crystallization solvent: methanol, a yellow solid, yield 74%, mp 180–181 °C.

IR (KBr): 1649, 1606, 1562, 1459, 1329, 1206, 1122, 1060, 1005.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (s, 1H, H-4), 7.84 (d, 1H, J = 16.1 Hz, H-β), 7.40 (d, 1H, J = 16.1 Hz, H-α), 7.28 (s, 1H, H-7), 6.31 (s, 2H, H-3′, H-5′), 5.63 (d, 1H, J = 11.4 Hz, H-2), 5.19 (d, 1H, J = 11.4 Hz, H-2), 4.09 (t, 1H, J = 6.3 Hz, OCH_2), 3.86 (s, 9H, 3 × OCH_3), 1.63 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 0.81 (t, 3H, J = 7.4 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 193.69, 164.21, 162.04, 153.73, 152.45, 137.28, 136.00, 133.05, 125.98, 113.27, 111.94, 105.36, 91.96, 91.68, 69.47, 56.72, 56.28, 31.18, 19.23, 14.23.

EA: Calcd for $C_{23}H_{26}O_7S$: C, 61.87; H, 5.87; S, 7.18. Found: C, 61.63; H, 5.90; S, 6.99.

5-Acetyl-6-methoxy-3-oxidobenzo[*d*][1,3]oxathiole (**9**).

About 30% Hydrogen peroxide (5 mL) was added to a solution of 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiole (**8**) (18) (1.05 g, 5 mmol) in acetic acid (15 mL) and the mixture was stirred at r.t. for 1 h. The solution was diluted with water (100 mL) and extracted with methylene chloride (3 × 30 mL). The organic layer was washed with water (20 mL), dried (Na_2SO_4), evaporated to dryness, and the residue was crystallized from methanol to give a colorless solid, yield 46%, mp 157–158 °C.

IR (KBr): 1665, 1593, 1472, 1260, 1235, 1039.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.29 (s, 1H, H-4), 7.13 (s, 1H, H-7), 5.67 (d, 1H, J = 5.4 Hz, H-2), 5.31 (d, 1H, J = 5.4 Hz, H-2), 3.97 (s, 3H, OCH_3), 2.53 (s, 3H, $COCH_3$).

EA: Calcd for $C_{10}H_{10}O_4S$: C, 53.09; H, 4.46; S, 14.17. Found: C, 52.99; H, 4.40; S, 14.34.

General procedure for condensation of 5-acetyl-6-methoxy-3-oxidobenzo[d][1,3]oxathiole (9) with benzaldehydes

A mixture of 5-acetyl-6-methoxy-3-oxidobenzo[*d*][1,3] oxathiole (**9**) (226 mg, 1 mmol), a benzaldehyde (1.2 mmol) and 5 N solution of NaOH (0.5 mL) in ethanol (10 mL) was stirred in darkness for 3–4 h, the formed solid was filtered off, washed with water and ethanol, and crystallized.

(*E*)-1-(6-Methoxy-3-oxidobenzo[*d*][1,3]oxathiol-5-yl)-3-phenylprop-2-en-1-one (**5a**), crystallization solvent: methanol, a colorless solid, yield 41%, mp 155–157 °C.

IR (KBr): 1651, 1603, 1573, 1469, 1245, 1068, 1037, 769.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.27 (s, 1H, H-4), 7.76 (m, 2H, H-2′, H-6′), 7.57 (d, 1H, J = 16.1 Hz, H-β), 7.47 (m, 4H, H-α, H-3′, H-4′, H-5′), 7.17 (s, 1H, H-7), 5.69 (d, 1H, J = 11.2 Hz, H-2), 5.33 (d, 1H, J = 11.2 Hz, H-2), 3.96 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.37, 164.62, 164.42, 143.48, 135.26, 131.26, 130.39, 129.70, 129.29, 127.15, 125.28, 123.65, 97.39, 93.37, 57.58.

EA: Calcd for $C_{17}H_{14}O_4S$: C, 64.95; H, 4.49; S, 10.20. Found: C, 65.06; H, 4.45; S, 10.17.

(*E*)-3-(3-Chlorophenyl)-1-(6-methoxy-3-oxidobenzo[*d*][1,3]oxathiol-5-yl)prop-2-en-1-one (**5b**), crystallization solvent: methanol, a cream solid, yield 52%, mp 165–167 °C.

IR (KBr): 1645, 1599, 1568, 1465, 1418, 1304, 1246, 1064.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.29 (s, 1H, H-4), 7.88 (s, 1H, H-2′), 7.74 (d, 1H, J = 7.3 Hz, H-6′), 7.56 (d, 1H, J = 16.8 Hz, H-β), 7.52 (d, 1H, J = 16.8 Hz, H-α), 7.48 (m, 2H, H-4′, H-5′), 7.17 (s, 1H, H-7), 5.69 (d, 1H, J = 11.2 Hz, H-2), 5.33 (d, 1H, J = 11.2 Hz, H-2), 3.96 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.20, 164.73, 164.53, 141.62, 137.60, 134.46, 131.45, 130.75, 130.52, 128.82, 128.54, 127.81, 125.11, 123.64, 97.39, 93.40, 57.60.

EA: Calcd for $C_{17}H_{13}ClO_4S$: C, 58.54; H, 3.76; S, 9.19. Found: C, 58.16; H, 3.97; S, 8.96.

(*E*)-1-(6-Methoxy-3-oxidobenzo[*d*][1,3]oxathiol-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**5c**), crystallization solvent: ethanol, a cream solid, yield 53%, mp 180–182 °C.

IR (KBr): 1650, 1597, 1571, 1510, 1255, 1174, 1066, 827.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.22 (s, 1H, H-4), 7.71 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.52 (d, 1H, J = 16.1 Hz, H-β), 7.30 (d, 1H, J = 16.1 Hz, H-α), 7.15 (s, 1H, H-7), 7.00 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 5.67 (d, 1H, J = 11.7 Hz, H-2), 5.31 (d, 1H, J = 11.7 Hz, H-2), 3.94 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.40, 164.40, 164.23, 162.00, 143.72, 132.55, 131.18, 130.12, 127.82, 125.60, 124.82, 123.51, 115.18, 97.32, 93.33, 57.50, 56.06.

EA: Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68; S, 9.31. Found: C, 62.74; H, 4.85; S, 9.12.

5-Acetoxy-3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiole (**11**).

About 30% Hydrogen peroxide (1 mL) was added to a solution of 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiole (**8**) (18) (210 mg, 1 mmol) in acetic acid (3 mL) and the mixture was stirred at 50 °C for 24 h. The precipitate was filtered off, washed with water, dried, and crystallized from 2-methoxyethanol to give a colorless solid, yield 139 mg (62%), mp 240–243 °C.

IR (KBr): 1766, 1612, 1491, 1447, 1308, 1271, 1125, 910.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.71 (s, 1H, H-4), 7.10 (s, 1H, H-7), 5.42 (s, 2H, H-2), 3.86 (s, 3H, OCH_3), 2.27 (s, 3H, $OCOCH_3$).

EA: Calcd $C_{10}H_{10}O_6S$: Found: C, 46.48; H, 3.85; S, 12.35.

5-Acetyl-3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiole (**12**).

A mixture of 5-acetyl-6-methoxybenzo[*d*][1,3]oxathiole (**8**) (630 mg, 3 mmol), potassium permanganate (1.92 g, 12 mmol), methylene chloride (18 mL) and water (36 mL) was vigorously stirred at rt for 2 h. Next, the mixture was decolorized by addition of aqueous sodium dithionite (~1.5 g) solution, the organic layer was separated, and the water layer was washed with methylene chloride (2 × 30 mL). The combined organic layers were washed with water (20 mL), dried (Na_2SO_4), and evaporated. Crystallization of the residue from methanol gave a colorless solid, yield 541 mg (74%), mp 205–208 °C.

IR (KBr): 1674, 1599, 1473, 1309, 1264, 1236, 1129, 1046.

^{1}H NMR (200 MHz, DMSO-d_6): δ 7.98 (s, 1H, H-4), 7.09 (s, 1H, H-7), 5.46 (s, 2H, H-2), 3.95 (s, 3H, OCH_3), 2.51 (s, 3H, $COCH_3$).

EA: Calcd for $C_{10}H_{10}O_5S$: C, 49.58; H, 4.16; S, 13.24. Found: C, 49.53; H, 4.10; S, 13.44.

5-Acetyl-3,3-dioxido-6-hydroxybenzo[*d*][1,3]oxathiole (**14**).

A mixture of 5-acetyl-6-hydroxybenzo[*d*][1,3]oxathiole (**13**) (19) (294 mg, 1.5 mmol), potassium permanganate (960 mg, 6 mmol), tetrabutylammonium bromide (120 mg, 0.4 mmol), methylene chloride (5 mL) and water (20 mL) was vigorously stirred at rt for 1.5 h. Next, the mixture was decolorized by addition of aqueous sodium dithionite solution, the organic layer was separated, and the water layer was washed with methylene chloride (2 × 20 mL). The combined organic layers were washed with water

(20 mL), dried (Na_2SO_4), and evaporated. The residue was purified on silica gel chromatographic column in methylene chloride to give a colorless solid, yield 154 mg (43%).

IR (KBr): 3435, 1647, 1616, 1590, 1477, 1375, 1310, 1264, 1135, 1048.

^{1}H NMR (200 MHz, DMSO-d_6): δ 12.85 (bs, 1H, OH), 8.49 (s, 1H, H-4), 6.81 (s, 1H, H-7), 5.45 (s, 2H, H-2), 2.67 (s, 3H, $COCH_3$).

5-Acetyl-3,3-dioxido-6-(2-morpholinoethoxy)benzo[*d*][1,3] oxathiole (**15**).

A mixture of 5-acetyl-3,3-dioxido-6-hydroxybenzo[*d*][1,3] oxathiole (**14**) (360 mg, 1.58 mmol), 4-(2-chloroethyl)morpholine hydrochloride (440 mg, 2.37 mmol), potassium carbonate (830 mg, 6 mmol) in dry DMF (4 mL) was stirred at 60 °C for 4 h. The cooled reaction mixture was diluted with water (100 mL) extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated. The residue was treated with ethyl ether, the formed solid was filtered off and crystallized from methanol to give a colorless solid, yield 46%.

IR (KBr): 1672, 1607, 1457, 1312, 1261, 1238, 1136, 1115, 1049.

^{1}H NMR (200 MHz, DMSO-d_6): δ 7.97 (s, 1H, H-4), 7.14 (s, 1H, H-7), 5.47 (s, 2H, H-2), 4.30 (t, 2H, J = 5.4 Hz, OCH_2), 3.56 (m, 4H, $O(CH_2)_2$), 2.77 (t, 2H, J = 5.4 Hz, NCH_2), 2.58 (s, 3H, $COCH_3$), 2.47 (m, 4H, $N(CH_2)_2$).

EA: Calcd for $C_{15}H_{19}NO_6S$: C, 52.77; H, 5.61; N, 4.10; S, 9.39. Found: 52.55; H, 5.50; N, 4.22; S, 9.19.

6-Acetyl-3,3-dioxido-5-methoxybenzo[*d*][1,3]oxathiole (**18**).

The reaction was done analogously to synthesis of compound **14**. The product was crystallized from methanol to give a colorless solid, yield 64%, mp. 162–165 °C.

IR (KBr): 1680, 1476, 1404, 1312, 1169, 1142, 1030.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.67 (s, 1H, H-4), 7.36 (s, 1H, H-7), 5.42 (s, 2H, H-2), 3.92 (s, 3H, OCH_3), 2.53 (s, 3H, $COCH_3$).

EA: Calcd for $C_{10}H_{10}O_5S$: C, 49.58; H, 4.16; S, 13.24. Found: C, 49.60; H, 4.05; S, 13.02.

6-Acetyl-3,3-dioxido-5-propoxybenzo[*d*][1,3]oxathiole (**19**).

The reaction was done analogously to synthesis of compound **29**. The product was crystallized from ethanol to give a colorless solid, yield 46%, mp. 170–172 °C.

IR (KBr): 1676, 1463, 1321, 1170, 1141.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.63 (s, 1H, H-4), 7.34 (s, 1H, H-7), 5.40 (s, 2H, H-2), 4.09 (t, 2H, J = 6.3 Hz, OCH_2), 2.54 (s, 3H, $COCH_3$), 1.75 (m, 2H, CH_2), 0.98 (t, 3H, J = 7.3 Hz, CH_3).

EA: Calcd for $C_{12}H_{14}O_5S$: C, 53.32; H, 5.22; S, 11.86. Found: C, 53.20; H, 5.18; S, 11.51.

General procedure for condensation of acetyl-3,3-dioxidobenzo[d][1,3]oxathiole derivatives 12, 15, 18, and 19 with benzaldehydes

A mixture of suitable sulfone (1 mmol), a benzaldehyde (1.2 mmol) and 5 N solution of NaOH or KOH (0.5 mL) in ethanol (10 mL) was stirred in darkness for several hours (3–20), the formed solid was filtered off, washed with water and ethanol, and crystallized.

(*E*)-1-(3,3-Dioxido-5-methoxybenzo[*d*][1,3]oxathiol-6-yl)-3-phenylprop-2-en-1-one (**6a**), crystallization solvent: methanol, a colorless solid, yield 66%, mp 161–162 °C.

IR (KBr): 1639, 1476, 1408, 1303, 1172, 1151, 1028.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.77 (dd, 2H, J_1 = 7.8 Hz, J_2 = 2.0 Hz, H-2′, H-6′), 7.69 (s, 1H, H-4), 7.42–7.48 (m, 4H, H-3′, H-4′, H-5′, H-β), 7.38 (s, 1H, H-7), 7.25 (d, 1H, J = 16.1 Hz, H-α), 5.45 (s, 2H, H-2), 3.87 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.82, 153.63, 150.76, 146.18, 136.90, 134.86, 131.70, 129.71, 129.55, 127.07, 125.65, 115.87, 105.22, 83.77, 57.69.

EA: Calcd for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; S, 9.71. Found: C, 61.60; H, 4.45; S, 9.52.

(*E*)-3-(3-Chlorophenyl)-1-(3,3-dioxido-5-methoxybenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (**6b**), crystallization solvent: methanol, a cream solid, yield 82%, mp 170–172 °C.

IR (KBr): 1644, 1597, 1475, 1410, 1308, 1150, 1028.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.90 (s, 1H, H-2′), 7.75 (d, 1H, J = 7.8 Hz, H-6′), 7.69 (s, 1H, H-4), 7.44–7.52 (m, 3H, H-4′, H-5′, H-β), 7.38 (s, 1H, H-7), 7.33 (d, 1H, J = 16.1 Hz, H-α), 5.44 (s, 2H, H-2), 3.87 (s, 3H, OCH_3).

EA: Calcd for $C_{17}H_{13}ClO_5S$: C, 55.97; H, 3.59; S, 8.79. Found: C, 55.81; H, 3.57; S, 8.53.

(*E*)-1-(3,3-Dioxido-5-methoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6c**) crystallization solvent: methanol, a colorless solid, yield 95%, mp 197–199 °C.

IR (KBr): 1633, 1598, 1475, 1410, 1313, 1260, 1171, 1028.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.72 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.66 (s, 1H, H-4), 7.40 (d, 1H, J = 16.1 Hz, H-β), 7.34 (s, 1H, H-7), 7.09 (d, 1H, J = 16.1 Hz, H-α), 6.98 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 5.44 (s, 2H, H-2), 3.86 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

EA: Calcd for $C_{18}H_{16}O_6S$: C, 59.99; H, 4.47; S, 8.90. Found: C, 59.83; H, 4.41; S, 8.90.

(*E*)-1-(3,3-Dioxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**6d**), crystallization solvent: methanol, a cream solid, yield 46%, mp 148–149 °C.

IR (KBr, cm^{-1}): 1665, 1599, 1570, 1513, 1423, 1312, 1155, 1032, 825.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.72 (d, 2H, J = 8.6 Hz, H-2′, H-6′), 7.66 (s, 1H, H-4), 7.44 (d, 1H, J = 16.0 Hz, H-β), 7.34 (s, 1H, H-7), 7.15 (d, 1H, J = 16.0 Hz, H-α), 7.00 (d, 2H, J = 8.6 Hz, H-3′, H-5′), 5.44 (s, 2H, H-2), 4.08 (t, 2H, J = 6.2 Hz, OCH_2), 3.82 (s, 3H, OCH_3), 1.64 (m, 2H, CH_2), 0.86 (t, 3H, J = 7.3 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.54, 162.27, 152.97, 150.66, 145.83, 137.43, 131.38, 127.47, 125.56, 124.76, 115.85, 115.20, 105.75, 83.75, 71.44, 56.08, 22.51, 11.05.

EA: Calcd for $C_{20}H_{20}O_6S$: C, 61.84; H, 5.19; S, 8.25. Found: C, 61.96; H, 5.23; S, 8.28.

(*E*)-1-(3,3-Dioxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**6f**), crystallization from 2-methoxyethanol – ethanol mixture, a cream solid, yield 62%, mp 174–175 °C.

IR (KBr, cm^{-1}): 1665, 1602, 1580, 1507, 1418, 1307, 1154, 1132, 1026.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.68 (s, 1H, H-4), 7.41 (d, 1H, J = 16.1 Hz, H-β), 7.36 (s, 1H, H-7), 7.30 (d, 1H, J = 16.1 Hz, H-α), 7.12 (s, 2H, H-2′, H-6′), 5.45 (s, 2H, H-2), 4.09 (t, 2H, J = 6.2 Hz, OCH_2), 3.82 (s, 6H, 2 × OCH_3), 3.71 (s, 3H, OCH_3), 1.66 (m, 2H, CH_2), 0.88 (t, 3H, J = 7.4 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.79, 153.76, 153.01, 150.62, 146.19, 140.52, 137.34, 130.46, 126.57, 125.59, 115.79, 106.99, 105.79, 83.76, 71.48, 60.81, 56.71, 22.54, 11.12.

EA: Calcd for $C_{22}H_{24}O_8S$: C, 58.92; H, 5.39; S, 7.15. Found: C, 58.62; H, 5.41; S, 7.03.

(*E*)-1-(3,3-Dioxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(3-fluoro-4-methoxyphenyl)prop-2-en-1-one (**6g**), crystallization from 2-methoxyethanol – ethanol mixture, a cream solid, yield 57%, mp 178–179 °C.

IR (KBr, cm^{-1}): 1654, 1578, 1512, 1313, 1278, 1142, 1125, 1030.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.75 (dd, 1H, J_1 = 12.7 Hz, J_2 = 1.8 Hz, H-2′), 7.67 (s, 1H, H-4), 7.57 (d, 1H, J = 8.6 Hz, H-6′), 7.42 (d, 1H, J = 16.1 Hz, H-β), 7.35 (s, 1H, H-7), 7.22 (t, 1H, J = 8.6 Hz, H-5′), 7.21 (d, 1H, J = 16.1 Hz, H-α), 5.44 (s, 2H, H-2), 4.08 (t, 2H, J = 6.2 Hz, OCH_2), 3.90 (s, 3H, OCH_3), 1.64 (m, 2H, CH_2), 0.86 (t, 3H, J = 7.3 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.61, 153.15, 153.01, 151.20, 150.63, 150.12, 150.04, 144.71, 137.24, 128.14, 128.08, 127.51, 126.13, 125.65, 115.93, 115.86, 115.78, 114.53, 105.78, 83.75, 71.45, 56.86, 22.51, 11.03.

EA: Calcd for $C_{20}H_{19}FO_6S$: C, 59.10; H, 4.71; S, 7.89. Found: C, 58.69; H, 4.60; S, 7.82.

(*E*)-1-(3,3-Dioxido-6-methoxybenzo[*d*][1,3]oxathiol-5-yl)-3-phenylprop-2-en-1-one (**7a**), crystallization from methanol, a cream solid, yield 66%, mp 180–183 °C.

IR (KBr, cm^{-1}): 1656, 1606, 1580, 1301, 1129, 1074.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.00 (s, 1H, H-4), 7.76 (m, 2H, H-2′, H-6′), 7.55 (d, 1H, J = 15.6 Hz, H-β), 7.40–7.47 (m, 4H, H-3′, H-4′, H-5′, H-α), 7.14 (s, 1H, H-7), 5.49 (s, 2H, H-2), 3.95 (s, 3H, OCH_3).

Calcd for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; S, 9.71. Found: C, 61.90; H, 4.24; S, 9.51.

(*E*)-3-(4-Bromophenyl)-1-(3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiol-5-yl)prop-2-en-1-one (**7b**), crystallization from 2-methoxyethanol, a cream solid, yield 59%, mp 233–234 °C.

IR (KBr, cm^{-1}): 1608, 1584, 1298, 1258, 1130, 1069.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.01 (s, 1H, H-4), 7.74 (d, 2H, J = 8.5 Hz, H-2′, H-6′), 7.64 (d, 2H, J = 8.5 Hz, H-3′, H-5′), 7.53 (d, 1H, J = 15.93 Hz, H-β), 7.45 (d, 1H, J = 15.93 Hz, H-α), 7.14 (s, 1H, H-7), 5.49 (s, 2H, H-2), 3.94 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.18, 164.60, 161.56, 142.75, 134.47, 132.65, 131.29, 127.61, 126.01, 124.74, 124.26, 116.01, 99.56, 83.89, 57.82.

Calcd for $C_{17}H_{13}BrO_5S$: C, 49.89; H, 3.20; S, 7.84. Found: C, 49.67; H, 3.28; S, 7.71.

(*E*)-3-(3-Chlorophenyl)-1-(3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiol-5-yl)prop-2-en-1-one (**7c**), crystallization from methylene chloride – methanol mixture, a cream solid, yield 74%, mp 192–195 °C.

IR (KBr, cm^{-1}): 1657, 1608, 1579, 1476, 1311, 1257, 1131, 1072.

^{1}H NMR (500 MHz, DMSO-d_6): δ 8.03 (s, 1H, H-4), 7.89 (s, 1H, H-2′), 7.74 (d, 1H, H-6′), 7.54 (d, 1H, J = 16.1 Hz, H-β), 7.44–7.52 (m, 3H, H-4′, H-5′, H-α), 7.14 (s, 1H, H-7), 5.49 (s, 2H, H-2), 3.94 (s, 3H, OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.21, 164.64, 161.58, 142.38, 137.46, 134.47, 131.43, 130.88, 128.90, 128.30, 127.93, 125.98, 124.28, 115.99, 99.54, 83.89, 57.83.

EA: Calcd for $C_{17}H_{13}ClO_5S$: C, 55.97; H, 3.59; S, 8.79. Found: C, 55.67; H, 3.57; S, 8.93.

(*E*)-1-(3,3-Dioxido-6-methoxybenzo[*d*][1,3]oxathiol-5-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (**7d**), crystallization from toluene, a cream solid, yield 54%, mp 210–212 °C.

IR (KBr, cm^{-1}): 1650, 1601, 1570, 1511, 1304, 1251, 1130, 1074, 827.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.96 (s, 1H, H-4), 7.72 (d, 2H, J = 8.8 Hz, H-2′,H-6′), 7.50 (d, 1H, J = 16.1 Hz, H-β), 7.26 (d, 1H, J = 16.1 Hz, H-α), 7.13 (s, 1H, H-7), 7.00 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 5.48 (s, 2H, H-2), 3.93 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3).

Calcd for $C_{18}H_{16}O_6S$: C, 59.99; H, 4.47; S, 8.90. Found: C, 60.00; H, 4.46; S, 8.81.

(*E*)-3-(3,4-Dimethoxyphenyl)-1-(3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiol-5-yl)prop-2-en-1-one (**7e**), crystallization from 2-methoxyethanol, an yellow solid, yield 54%, mp 218–220 °C.

IR (KBr, cm^{-1}): 1646, 1610, 1513, 1302, 1273, 1132, 1079, 1022.

^{1}H NMR (500 MHz, DMSO-d_6): δ 7.94 (s, 1H, H-4), 7.45 (d, 1H, J = 16.0 Hz, H-β), 7.36 (s, 1H, H-2′), 7.31 (d, 1H, J = 8.3 Hz, H-6′), 7.29 (d, 1H, J = 16.0 Hz, H-α), 7.13 (s, 1H, H-7), 7.01 (d, 1H, J = 8.3 Hz, H-5′), 5.48 (s, 2H, H-2), 3.92 (s, 3H, OCH_3), 3.81 (s, 6H, 2 × OCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 190.84, 164.24, 161.16, 151.99, 149.65, 145.27, 127.87, 126.65, 124.96, 124.04, 123.57, 115.82, 112.30, 111.59, 99.45, 83.86, 57.69, 56.31, 56.29.

Calcd for $C_{19}H_{18}O_7S$: C, 58.45; H, 4.65; S, 8.21. Found: C, 58.63; H, 4.55; S, 8.00.

(*E*)-3-(3-Fluoro-4-methoxyphenyl)-1-(6-(2-morpholinoethoxy)-3,3-dioxidobenzo[*d*][1,3]oxathiol-5-yl)prop-2-en-1-one methanesulfonate hydrate (**7f**). Product of condensation (free base) was obtained after crystallization from chloroform – methanol mixture, as an yellow solid. The product (0.5 mmol) was dissolved in a solution of methanesulfonic acid (1 mmol) in methylene chloride (2 mL). The formed clear solution was diluted with ethyl ether, the precipitated solid was filtered off, and washed with ethyl ether, to give a hygroscopic yellow solid, yield 56%, mp 90 °C – softening, 145–150 °C melting.

IR (KBr, cm^{-1}): 3442, 1670, 1606, 1513, 1305, 1192, 1127, 1073.

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.91 (bs, 1H, NH^+), 8.09 (s, 1H, H-4), 7.81 (d. 1H, J = 12.7 Hz, H-2′), 7.59 (d, 1H, J = 8.3 Hz, H-6′), 7.50 (d, 1H, J = 16.1 Hz, H-β), 7.36 (d, 1H, J = 16.1 Hz, H-α), 7.23 (m, 2H, H-5′, H-7), 5.51 (s, 2H, H-2), 4.56 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 3.84 (m, 2H, CH_2), 3.60 (m, 4H, 2 × CH_2), 3.45 (m, 2H, CH_2), 3.12 (m, 2H, CH_2).

^{13}C NMR (for a free base) (126 MHz, DMSO-d_6) δ 190.32, 163.66, 161.35, 153.19, 151.24, 149.92, 149.83, 142.95, 128.45, 128.40, 127.47, 126.44, 126.21, 124.07, 116.06, 115.68, 115.53, 114.53, 100.14, 83.86, 68.34, 66.70, 57.32, 56.88, 54.18.

Calcd for $C_{23}H_{24}FNO_7S \times CH_3SO_3H \times H_2O$: Elemental Analysis: Found: C, 48.37; H, 5.31; N, 2.09; S, 10.64.

(*E*)-1-(3,3-Dioxido-5-propoxybenzo[*d*][1,3]oxathiol-6-yl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (**6e**). A solution of Oxone® (20 g) in water (80 mL) was added dropwise, during 2 h, to a stirred, boiling suspension of (*E*)-3-(3-hydroxy-4-methoxyphenyl)-1-(5-propoxybenzo[*d*][1,3]oxathiol-6-yl)prop-2-en-1-one (12) (3.72 g, 10 mmol) in ethanol (400 mL). The obtained mixture was concentrated under vacuum and diluted with water. The solid was filtered off, dried and purified on silica gel column in methylene chloride – methanol 20:1 solution, and crystallized from ethanol to give an yellow solid, yield 2.77 g (68%), mp 162–164 °C.

IR (KBr, cm^{-1}): 3397, 1648, 1572, 1510, 1319, 1268, 1152, 1025.

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.25 (s, 1H, OH), 7.65 (s, 1H, H-4), 7.35 (m, 2H, H-β, H-7), 7.16 (m, 2H, H-2′, H-6′), 7.03 (d, 1H, J = 16.0 Hz, H-α), 6.98 (d, 1H, J = 8.2 Hz, H-5′), 5.44 (s, 2H, H-2), 4.08 (t, 2H, J = 5.8 Hz, OCH_2), 3.83 (s, 3H, OCH_3), 1.65 (m, 2H, CH_2), 0.87 (t, 3H, J = 7.6 Hz, CH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 192.29, 152.98, 151.28, 150.66, 147.40, 146.24, 137.40, 127.72, 125.60, 124.48,

122.92, 115.90, 114.95, 112.62, 105.77, 83.75, 71.46, 56.32, 22.53, 11.06.

EA: Calcd for $C_{20}H_{20}O_7S$: C, 59.40; H, 4.98; S, 7.93. Found: C, 59.12; H, 4.87; S, 7.68.

Biology

Cells, drugs, and reagents

Human non-small lung adenocarcinoma A549 cells, human colon carcinoma HCT-116 cells and human ovary carcinoma HeLa cells used in these studies were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). A549 cells were cultured in RPMI1640 medium, HCT-116 cells in McCoy's 5 A medium and HeLa cells in high glucose DMEM. All media were supplemented with 10% fetal bovine serum (FBS), antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), and cells were grown at 37 °C in a humidified 5% CO_2-95% air atmosphere.

AMG compounds were dissolved in DMSO and kept at −80 °C until use. Vinblastine, paclitaxel, colchicine were from SIGMA-Aldrich-Fluka (Poznan, Poland). Radiolabeled ^{3}H-colchicine, biotinylated colchicine as well as streptavidin-coated yttrium beads were from Perkin Elmer. Tubulin Polymerization Kit and Colchicine Site Competitive Assay Kit were from Cytoskeleton Inc. (Denver, CO, USA). Doxorubicin was from the Institute of Biotechnology and Antibiotics (Warsaw, Poland). All other reagents were of analytical grade from either SIGMA-Aldrich-Fluka or POCH, S.A.

Cytotoxicity assays *in vitro* on A549, HCT116 and HeLa cell lines

Multiwell (24–well) plates were seeded at 3×10^3 cells/well in RPMI1640 medium supplemented with 10% FBS and antibiotics (penicillin/streptomycin), and cells were allowed to attach overnight. Drugs were added to wells in 10 μL aliquots of 200-times concentrated drug solutions dissolved in DMSO in duplicates. Ten microliters of DMSO was added to the control wells. Cells were incubated with studied drugs for 72 h at 37 °C in a 5% CO_2 atmosphere. To all wells, 200 μL of MTT solution in PBS (4 mg/mL) was added and incubated further for 3 h at 37 °C. Absorbance at 540 nm was measured after solubilization of formazan crystals in 1 mL DMSO using a multiwell plate reader (Victor3V; Perkin Elmer Wallac, Waltham, MA, USA). Cytotoxicity was determined upon comparison to control cells.

Tubulin polymerization assay

The test was performed according to manufacturer instructions. Briefly, purified tubulin was diluted in the reaction buffer (80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 EGTA) containing 1 mM GTP. Studied compounds were added at respective concentrations and the kinetics of tubulin polymerization was determined by reading changes in 340 nm absorbance with multiplate reader during 1 h at 37 °C. Drug-response curves were normalized for buffer absorbance and drug concentrations inhibiting tubulin polymerization by 50% were calculated using SLIDEWRITE (Advanced Graphics Software, Inc., Rancho Santa Fe, CA, USA) program.

Colchicine site competition assay

The assays were carried out using CytoDYNAMIX Screen 15 kit (Cytoskeleton Inc.) according to manufacturer's description. Briefly, biotinylated tubulin was bound to streptavidin-coated yttrium SPA beads in G-PHEM buffer for 30 min at 4 °C and exposed to ^{3}H-colchicine in the absence or presence of studied compounds. Radioactivity of ^{3}H-colchicine bound to tubulin was read using MicroBeta2 scintillation counter (Perkin Elmer Wallac).

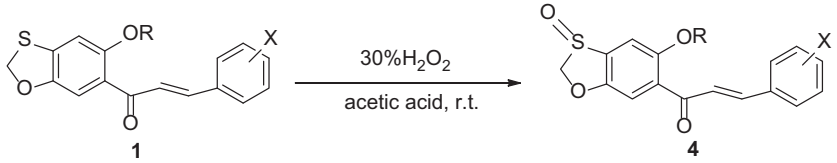
Results and Discussion

Chemistry

Mono-oxidized derivatives of chalcones (**4**) were prepared by oxidation of related chalcones (**1**) (18) with hydrogen peroxide in acetic acid at room temperature (Scheme 1). The obtained products are listed in Table 1.

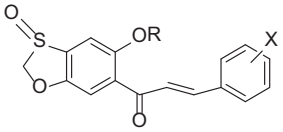
Isomeric sulfoxides **5** were prepared by oxidation of acetophenone **8** (19) and condensation of the obtained sulfoxide **9** with suitable benzaldehydes (Scheme 2). The obtained products are listed in Table 2.

An attempt of preparation of the starting 6-acetyl-5-methoxy-3,3-dioxidobenzo[*d*][1,3]oxathiole (**12**) by oxidation of compound **10** (19) with hydrogen peroxide at elevated temperature failed, as oxidation of the sulfur atom was accompanied by Bayer – Villiger rearrangement to give ester **11**. The desired sulfone **12** was obtained by



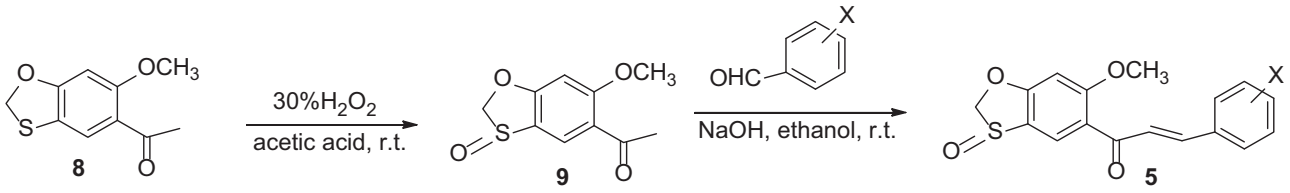
Scheme 1: Synthesis of 3-oxidobenzo[*d*][1,3]oxathiol-6-yl)-3-phenylprop-2-en-1-ones (**4**).

Table 1: The obtained S-monoxido derivatives of chalcone (**4**) and their cytotoxic activity



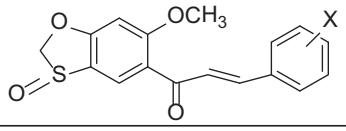
Comp. No., Comp. code	Substituent R	Substituent X	Cytotoxicity, IC_{50} [μM] ± SD			Influence on activity[a]
			A549	HCT116	HeLa	
4a, AMG-225	CH_3	–	0.404 ± 0.098	0.195 ± 0.059	0.129 ± 0.025	0.10
4b, AMG-224	CH_3	3-Cl	0.200 ± 0.008	0.104 ± 0.023	0.068 ± 0.017	–
4c, AMG-221	CH_3	4-OCH_3	0.033 ± 0.010	0.021 ± 0.004	0.014 ± 0.000	0.09
4d, AMG-304	CH_3	3-OH-4-OCH_3	0.010 ± 0.001	0.007 ± 0.000	0.005 ± 0.000	0.2
4e, AMG-302	CH_3	3-F-4-OCH_3	0.033 ± 0.003	0.033 ± 0.001	0.012 ± 0.000	0.12
4f, AMG-306	CH_3	2,4,6-triOCH_3	0.325 ± 0.100	0.447 ± 0.038	0.093 ± 0.000	1.0
4g, AMG-332	$CH_2CH_2CH_3$	3-OH-4-OCH_3	0.006 ± 0.002	0.002 ± 0.0005	0.002 ± 0.0005	0.27
4h, AMG-354	$CH_2CH_2CH_3$	3-OPO_3Na_2-4-OCH_3	0.010 ± 0.003	0.003 ± 0.001	0.003 ± 0.001	0.08
4i, AMG-335	$CH_2CH_2CH_3$	2,4,6-triOCH_3	0.182 ± 0.116	0.171 ± 0.051	0.038 ± 0.011	0.03
4j, AMG-309	$CH_2CH_2CH_3$	3-F-4-OCH_3	0.009 ± 0.003	0.006 ± 0.001	0.005 ± 0.000	0.06
4k, AMG-339	$(CH_2)_3CH_3$	3,4,5-triOCH_3	2.28 ± 0.232	1.92 ± 0.222	2.25 ± 0.258	0.19
4l, AMG-340	$(CH_2)_3CH_3$	2,4,6-triOCH_3	0.072 ± 0.007	0.039 ± 0.014	0.018 ± 0.004	0.02
Doxorubicin			0.124 ± 0.006	0.069 ± 0.027	0.154 ± 0.017	
CA4			0.008 ± 0.003	0.004 ± 0.001	0.003 ± 0.000	
Pacilitaxel			0.006 ± 0.002	0.004 ± 0.001	0.008 ± 0.003	

SD, standard deviation.
[a]Influence of oxidation on cytotoxic activity against A549 cells, calculated as a ratio of IC_{50} of oxidized analog **4** to IC_{50} of unoxidized form **1** (18).



Scheme 2: Synthesis of 3-oxidobenzo[*d*][1,3]oxathiol-5-yl)-3-phenylprop-2-en-1-ones (**5**).

Table 2: The obtained S-monoxido derivatives of chalcone (**5**) and their cytotoxic activity

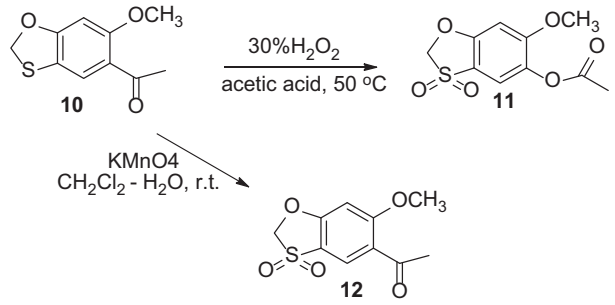


Comp. No, Comp. code	Substituent X	Cytotoxicity, IC_{50} [μM] ± SD			Influence on activity[a]
		A549	HCT116	HeLa	
5a, AMG-212	–	4.81 ± 0.56	2.40 ± 0.57	4.29 ± 0.69	1.2
5b, AMG-211	3-Cl	1.56 ± 0.09	0.960 ± 0.040	1.63 ± 0.14	0.8
5c, AMG-186	4-OCH_3	6.39 ± 0.06	4.90 ± 0.23	3.57 ± 0.06	18.1

SD, standard deviation.
[a]Influence of oxidation on cytotoxic activity against A549 cells, calculated as a ratio of IC_{50} of oxidized analog **5** to IC_{50} of unoxidized form **2** (19).

oxidation with potassium permanganate under phase-transfer conditions (Scheme 3).

Similar oxidation of 5-acetyl-6-hydroxybenzo[*d*][1,3]oxathiole (**13**) (19) gave the related dioxide **14**. It was further found that compound **14** could be obtained also by a prolonged (2–3 days) oxidation with 30% H_2O_2 – acetic acid at room temperature. Condensation of **14** with 4-(2-chloroethyl)morpholine gave compound **15** (Scheme 4).



Scheme 3: Synthesis of 5-acetyl-3,3-dioxido-6-methoxybenzo[*d*][1,3]oxathiole (**12**).

Oxidation with potassium permanganate was utilized also for preparation of sulfones **18** and **19** (Scheme 5).

The described above sulfones **12**, **15**, **18** and **19** were condensed with benzaldehydes under typical alkaline conditions to give related chalcones (Scheme 6, Tables 3 and 4).

Surprisingly, the method failed in case of condensation of 3-hydroxy-4-methoxybenzaldehyde with acetophenone **19**. The reaction was relatively slow, and a prolonged time was required to achieve an acceptable degree of transformation of **19**. As evidenced by TLC, the initially formed chalcone **6e** reacted further to give a mixture of unidentified, colored products of low Rf, and the chalcone was isolated with yields below 10%. It could be speculated that the side products were formed through reactions of carbanions formed at carbon α to the sulfone group. For these reasons, the chalcone **6e** was prepared by Oxone® oxidation of related chalcone **1** (R = $OCH_2CH_2CH_3$, X = 3-OH-4-OCH_3).

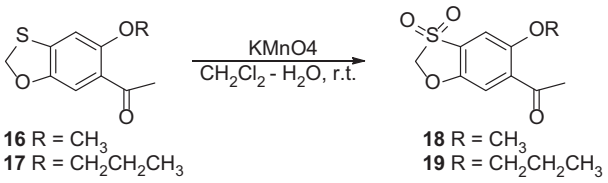
Cytotoxic Activity *In Vitro*

Evaluation of cytotoxic activity of the prepared compounds against three tumor cell lines using the MTT test (Tables 1–4) demonstrated strong influence of oxidation on activity (Tables 1–4, last columns). Oxidation of sulfur atom in chalcones **1** to sulfoxides (chalcones **4**) resulted in 5–10 times increase in activity, two of the compounds demonstrated activity at nanomolar level (**4g** – IC_{50} = 0.006 μM; **4j** – IC_{50} = 0.009 μM; all IC_{50} values used in this discussion were measured in A549 cells). However, for the 2,4,6-trimethoxy derivative **4f**, the IC_{50} value was practically identical as for the parent compound. Oxidation of the sulfoxides **4** to sulfones **6** also influenced significantly the activity. For ring B unsubstituted derivatives (compound **4a** versus compound **6a**) activity dropped by a factor of 4, and for 4-OCH_3 derivatives (compound **4c** versus compound **6c**) by a factor of 9. Yet, an opposite effect was observed for 3-OH-4-OCH_3 derivatives (compound **4g** versus compound **6e**) as oxidation to sulfone resulted in twofold increase in activity. Generally, the tendency to increase in activity prevailed, and three of the chalcones **6** demonstrated activity at nanomolar level (**6d** – IC_{50} = 0.004 μM; **6e** – IC_{50} = 0.003 μM, and **6g** – IC_{50} = 0.007 μM).

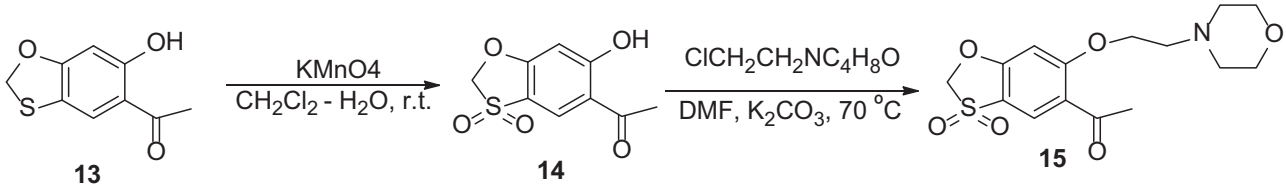
For chalcones **2**, **5,** and **7**, bearing the sulfur atom in position *meta* to the carbonyl group, the influence of oxidation on cytotoxic activity was not as clear as for the their isomers **1**, **4,** and **6** discussed above. For ring B unsubstituted compounds **5a** and **7a**, oxidation almost did not influence the activity. However, for 4-OCH_3 derivatives **5c** and **7d**, a sharp drop in activity was observed. Interestingly, the effect was opposite to the one observed for isomer with the sulfur atom *para* to the carbonyl group.

Interaction with Tubulin

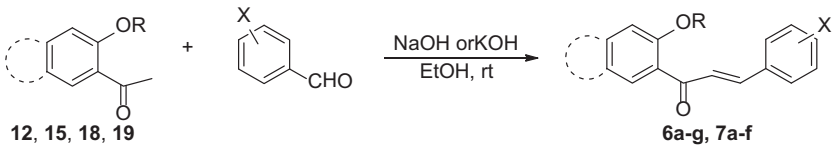
According to our early observations (19,23) the oxathiole-fused chalcones, similarly as many other chalcones (24–26) exhibit cytotoxic activity due to their influence on polymerization of tubulin. It was of interest whether the mechanism is also in operation in case of the oxidized derivatives? To check it, inhibition of tubulin polymerization and binding of the compounds at the colchicine site of tubulin were studied experimentally (Table 5), and the results proved that the oxidized compounds also interact with tubulin.



Scheme 5: Synthesis of 6-acetyl-5-alkoxy-3,3-dioxidobenzo[*d*][1,3]oxathioles **18** and **19**.

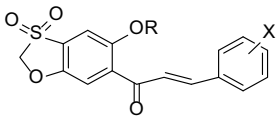


Scheme 4: Synthesis of 5-acetyl-3,3-dioxido-6-(2-morpholinoethoxy)benzo[*d*][1,3]oxathiole (**15**).



Scheme 6: Synthesis of chalcones **6a**–**g** and **7a**–**f**.

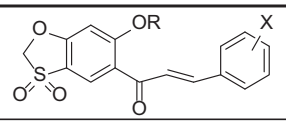
Table 3: *In vitro* cytotoxicity of the prepared chalcones of general structure **6**



Comp. No, Comp. code	Substituent R	Substituent X	Cytotoxicity, IC_{50} [μM] ± SD			Influence on activity[a]
			A549	HCT116	HeLa	
6a, AMG-229	CH_3	–	1.66 ± 0.43	0.94 ± 0.09	0.64 ± 0.02	0.4
6b, AMG-223	CH_3	3-Cl	1.08 ± 0.30	0.65 ± 0.07	0.51 ± 0.07	–
6c, AMG-228	CH_3	4-OCH_3	0.293 ± 0.014	0.036 ± 0.006	0.059 ± 0.020	0.8
6d, AMG-363	$CH_2CH_2CH_3$	4-OCH_3	0.004 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	–
6e, AMG-358	$CH_2CH_2CH_3$	3-OH-4-OCH_3	0.003 ± 0.001	0.001 ± 0.000	0.002 ± 0.0005	0.1
6f, AMG-371	$CH_2CH_2CH_3$	3,4,5-triOCH_3	1.59 ± 0.262	1.06 ± 0.014	1.55 ± 0.071	–
6g, AMG-370	$CH_2CH_2CH_3$	3′-F-4′-OCH_3	0.007 ± 0.002	0.002 ± 0.000	0.001 ± 0.000	0.05

SD, standard deviation.
[a]Influence of oxidation on cytotoxic activity against A549 cells, calculated as a ratio of IC_{50} of oxidized analog **6** to IC_{50} of unoxidized form **1** (18).

Table 4: *In vitro* cytotoxicity of the prepared chalcones of general structure **7**



Comp. No, Comp. code	Substituent R	Substituent X	Cytotoxicity, IC_{50} [μM] ± SD			Influence on activity[a]
			A549	HCT116	HeLa	
7a, AMG-213	CH_3	–	3.05 ± 0.42	2.34 ± 0.36	2.83 ± 0.13	0.7
7b, AMG-215	CH_3	4-Br	1.60 ± 0.14	1.43 ± 0.18	1.60 ± 0.01	0.5
7c, AMG-214	CH_3	3-Cl	1.07 ± 0.31	0.88 ± 0.20	1.10 ± 0.13	0.5
7d, AMG-192	CH_3	4-OCH_3	1.87 ± 0.14	0.91 ± 0.11	0.48 ± 0.02	5.3
7e, AMG-216	CH_3	3,4-diOCH_3	9.59 ± 1.99	11.64 ± 2.76	7.24 ± 0.80	8.0
7f, AMG-220ms	CH_2CH_2morpholine	3-F-4-OCH_3	6.86 ± 0.27	4.27 ± 0.17	6.19 ± 1.08	1.6

SD, standard deviation.
[a]Influence of oxidation on cytotoxic activity against A549 cells, calculated as a ratio of IC_{50} of oxidized analog **7** to IC_{50} of unoxidized form **2** (19).

Our earlier molecular modeling evaluation of interaction of selected chalcones **2** with tubulin indicated two preferential poses of the chalcones inside the colchicine binding site (19). The first one, referred further as 'A' was adopted by compounds **2** bearing alkoxy and hydroxy substituents in the hydrophobic ring B. The second pose, referred further as 'B', could be forced either by a presence of aminoalkoxy group at position 2′ of the ring A or by 'inappropriate' substitution pattern of ring B, especially by larger substituents, for example additional methoxy groups, in positions 3 and 5, which disturb the interactions which are characteristic for pose 'A'. Prompted by the results, we have performed a molecular modeling evaluation of influence of oxidation of the sulfur atom on interactions of the compounds with tubulin. As the detailed procedure used to build complexes of chalcones with

Table 5: Interaction of oxathiole-fused chalcones with tubulin

Entry	Comp. No, Comp. label	Inhibition of polymerization IC_{50} [μM]	Binding at the colchicine site IC_{50} [μM]
1	**4c**	4.55 ± 0.4	0.195
2	**6c**	7.35 ± 0.3	0.164
3	**4g**	2.80 ± 0.4	0.021
4	**4j**	1.96 ± 0.3	0.191
5	**4k**	17.38 ± 0.7	
6	**AMG-190**[a]	4.15 ± 0.3	0.108
7	Vinblastine	2.23 ± 0.3	
8	Combretastatin A4	1.73 ± 0.2	0.023

[a]Unoxidized analog of compounds **4c** and **6c** of general formula **1**; cytotoxic activity against A549: IC_{50} [μM] = 0.369 (19).

tubulin was already described (19), it was not repeated here. As before, we have used flexible ligand docking with the receptor held rigid, to assure comparability of the results. It has to be noticed, that due to substantial flexibility of both loops and side chains within the colchicine binding site, the method could result in some inaccuracy. Based on the analysis of resulting complexes, S-oxidation of chalcones bearing the sulfur atom in the position para to the carbonyl group (compounds **4** and **6**), and accepting pose A in the colchicine binding site (as described in Ref. 19), does not influence the mode of ligand binding. In this ligand pose there is enough room around the chalcone's sulfur atom, facing the beta strands 2 and 3 of the beta subunit's second domain, to accommodate the introduction of even two additional oxygen atoms (Figure 2 left). Thus not only, there are no nearby groups causing any steric hindrance, but additional oxygen atoms can form favorable interactions with peptide bond dipoles of both beta strands. All other important interactions, specific for chalcones bound in the colchicine binding site in the pose A, are preserved.

However, that is not the case of derivatives with ring B substituted in such a way, that binding in the pose B (as described in Ref. 19) is preferred. Since in the pose B, the orientation of the benzoxathiole ring is flipped, oxidized sulfur atom located in the para position (compounds **4** and **6**) does not face the mentioned two beta strands, but is placed in the proximity of the Cys241 residue what leads to similar interactions like in the chalcones bearing the sulfur atom in meta position (compounds **5** and **7**) described below.

Such ligands (S-oxidized chalcones bearing the sulfur atom in the position meta to the carbonyl group) exhibit some differences in the interaction pattern with the binding site with respect to their unoxidized counterparts. In this case the oxidized ligand's pose has to change slightly since the additional oxygen replaces the sulfur in the interaction with the Cys241 thiol group (Figure 2 right). Moreover, nearby side chain of Leu248 residue becomes the source of the steric hindrance. Thus, it seems that there should be significant difference in activity of both possible stereoisomers of respective sulfoxides. One with slightly disrupted geometry but still able to form the hydrogen bond with Cys241 and the other one deprived of this ability and heavily hindered by the Leu248 side chain. Unfortunately, the above suggestion cannot be verified experimentally since activities were measured for racemic mixtures only. Further oxidation, leading to sulfones, may surprisingly improve the binding slightly since, despite still present unfavorable interaction of one of the oxygen atoms with Leu248, the second oxygen atom can form favorable interaction with Cys241, and counterbalance the unfavorable one with Leu248.

As demonstrated in Tables 1 and 3 (last columns) there was a positive influence of oxidation of the sulfur atom on cytotoxic activity. It was of interest if there was any correlation among the following factors: cytotoxic activity of the studied compounds – binding to tubulin at the colchicine site – inhibition of tubulin polymerization – and results of docking to tubulin at the colchicine site?

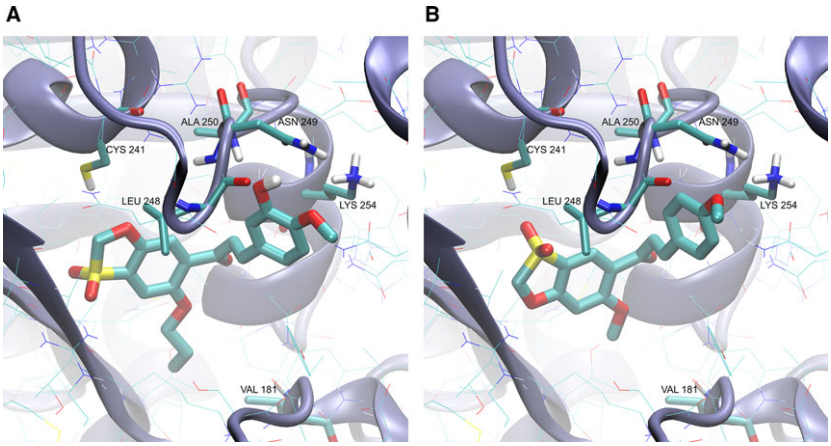


Figure 2: Docking poses of ligands **6e** (left) and **7d** (right). Ligands are drawn as thick sticks while important residues in the binding site as thinner sticks. Residue Val181 belongs to the α subunit of tubuline while all other shown residues belong to the subunit β.

Comparison of data from Table 5 (entries 1, 2, and 6) suggested that oxidation of the sulfur atom marginally decreased binding to tubulin and inhibition of polymerization while cytotoxic activity has changed in the opposite direction, for the sulfoxide **4c** even a ten times increase in the activity was observed. The data are not contradictory to conclusion from the molecular docking. Consequently, it could be concluded that most probably the observed influence of oxidation of the sulfur atom on cytotoxic activity was not exclusively related to interaction of the compounds with tubulin at the colchicine binding site as molecular target. It remains to be established whether the cytotoxic activity is influenced by an additional mechanism of the activity, differences in cell permeability, different metabolism, or still another cellular effect.

Conclusion

A new group of oxathiole-fused chalcone derivatives active at nanomolar level is described. It was found that oxidation of sulfur atom of the oxathiole-fused chalcones strongly influenced activity of the compounds, and that depending on relative position of the sulfur atom in the molecule, the activity could be either increased or diminished. Generally, for the most active isomers with sulfur atom para to the carbonyl group, oxidation led to increase of activity, while for isomers with sulfur atom meta to the carbonyl the activity dropped down. It was demonstrated that the cytotoxic activity of the compounds resulted, at least partly, from their interaction with tubulin at the colchicine binding site, but that the profound influence of oxidation of the sulfur atom on cytotoxic activity cannot be solely related to interaction of the compounds with tubulin.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. These data include ^{1}H and ^{13}C NMR spectra of the obtained chalcones.