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Diaryl Disulfides and Thiosulfonates as Combretastatin A-4 Analogues: Synthesis, Cytotoxicity and Antitubulin Activity

Rejane Gonçalves Diniz Khodyuk[a], **Ruoli Bai**[b], **Ernest Hamel**[b], **Estela Mariana Guimarães Lourenço**[a], **Euzébio Guimarães Barbosa**[c], **Adilson Beatriz**[a], **Edson dos Anjos dos Santos**[d], **Dênis Pires de Lima**[a,*]

[a]Universidade Federal de Mato Grosso do Sul, Instituto de Química, Laboratório LP4, Av. Filinto Müller, 1555, 79074-460, Campo Grande (MS), Brasil

[b]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, Frederick National Laboratory for Cancer Research (FNLCR), National Cancer Institute (NCI), National Institutes of Health, Frederick, MD 21702, USA

[c]Universidade Federal do Rio Grande do Norte, Departamento de Farmácia (DFAR), Grupo de Pesquisa em Química Computacional, Faculdade de Farmácia, 59012-570, Natal (RN), Brasil

[d]Universidade Federal de Mato Grosso do Sul, Instituto de Biociências (INBIO), Laboratório de Bioquímica, Cidade Universitária, 79070-900, Campo Grande (MS), Brasil

Abstract

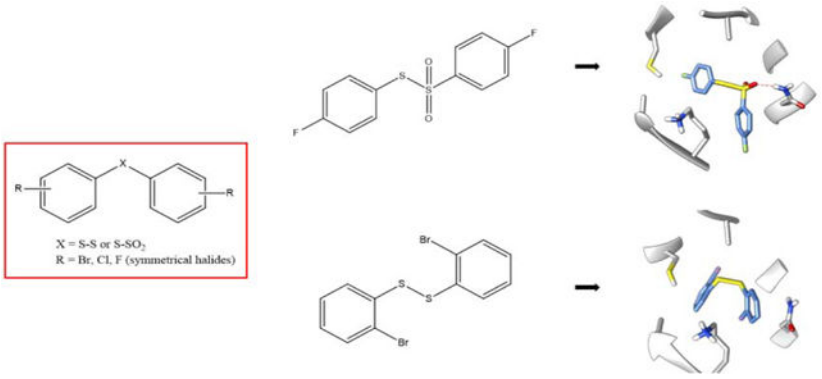
Diaryl disulfides and diaryl thiosulfonates were synthesized with the two phenyl rings of all compounds bearing identical halide substituents. Because of structural similarity to the potent antimitotic natural product combretastatin A-4 (CA-4), the compounds were examined for inhibition of tubulin polymerization, and the thiosulfonates were more active than the disulfides. The nine thiosulfonates had IC_{50} values ranging from 1.2 to 9.1 μM, as compared with 1.3 μM obtained with CA-4. The compounds thus ranged from equipotent with CA-4 to 7-fold less active. The nine disulfides had IC_{50} values ranging from 1.2 to 5.1 μM, as compared with 0.54 μM obtained with CA-4. The compounds thus ranged from less than half as active as CA-4 to over 9-fold less active. The most active members of each group, **2g** and **3c**, in the assembly assay were modeled into the colchicine site. Compound **3c** had significant hydrophobic interactions with β-tubulin residues CYS 241 and ALA 250, and its thiosulfonate bridge made a hydrogen bond with β-tubulin residue ASN 258. Compound **2g** had hydrophobic interactions with β-tubulin residues ALA 250, CYS 241 and ALA 254, but there was no significant interaction of the disulfide bridge with tubulin.

Graphical Abstract

[*]*Corresponding author*: Dênis Pires de Lima, denis.lima@ufms.br, Phone: (+55) 67 3345 3578, Fax: (+55) 67 3345 3552.

Conflicts of interest

The authors declare that the research was conducted in the absence of any conflicts of interest.



Keywords

Diaryl disulfides; Diaryl thiosulfonates; Combretastatin A-4; Tubulin; Cytotoxicity

Introduction

The attachment of halogen atoms to the structures of the potent antitubulin agent combretastatin A-4 (CA-4) led to analogues with equivalent antitubulin activity (Figure 1) [1]. Aryl thiosulfonates and their direct precursors aryl disulfides are known for their importance in life science, pharmaceutical potential, and food chemistry [2,3]. However, their biological actions, particularly antiproliferative effects, are still unexplored. As a part of our work on the synthesis of CA-4 analogues, we planned and synthesized diaryl disulfides and thiosulfonates bearing halides (Figure 1) in order to investigate their cytotoxicity against the MCF-7 tumor cell line and their potential to inhibit tubulin polymerization.

Methodologies aimed at direct conversion of halogenated thiols into the corresponding diaryl disulfides, followed by formation of diaryl thiosulfonates in reasonable yields, are limited to a few reports [4–7], making the search for new and effective procedures very desirable. Catalysts such as $AgNO_3/BF_3{\cdot}OEt_2$ [8] and $Al(H_2PO_4)_3$, HNO_3 [9] have been used for nitration of aromatic rings. Making use of these systems, our research group successfully achieved the synthesis of diaryl thiosulfonates from thiols.

Here we report an extension of the previous protocol that enabled us to perform a one-pot novel synthesis of different symmetrical halide-substituted diaryl disulfides and diaryl thiosulfonates directly from thiols with good yields. Since these compounds can be regarded as analogues of CA-4, they were evaluated for cytotoxicity against the human breast cancer cell line MCF-7 and for activity against purified tubulin.

Results and Discussion

Chemistry

Aspects of the previously described synthetic process were revised to obtain significantly better yields of the target compounds. After establishing optimal conditions, the diverse

halogenated thiols underwent ArS-SAr coupling. At room temperature, the reactions were slow, and diaryl disulfides were the predominant products using either system **A** or **B** (Table 1). Acetonitrile (ACN) efficiently increased the reaction rate, and heating reaction mixtures to ~ 85 °C resulted in oxidation of the disulfides to thiosulfonates. These compounds were obtained in high yields by the treatment of thiols with $Al(H_2PO_4)_3$-HNO_3 in ACN (System **B**, Table 1). Both protocols can also be applied to significantly larger reaction mixtures, and this scale-up also provided excellent yields.

The compounds were unambiguously characterized by NMR and IR spectra and by low and high-resolution mass spectrometry. The fluorinated diaryl disulfides **2a**-**2c** and diaryl thiosulfonates **3a**-**3c** presented the majority of ^{13}C NMR signals as doublets because of ^{1}J, ^{2}J and ^{3}J fluorine (spin ½) coupling with the respective carbons in both aromatic rings. Due to symmetry, the disulfides **2a**-**2i** showed a reduced number of chemical shift signals. When compared to starting materials, the compounds do not present SH group signals in the ^{1}H NMR spectra. NMR data were compared with those from the literature [7, 10–19] and the structures of the synthesized compounds were confirmed. In the IR spectra of **3a**-**3i**, the presence of the SO_2 group was verified by strong absorption bands in the regions 1350–1300 cm^{-1} and 1160–1120 cm^{-1}. The high-resolution mass spectra showed similar fragmentation patterns for the compounds bearing the same aromatic substituents, allowing ready assignment of mass units.

Biological evaluation

The compounds described here were evaluated for effects on the growth of MCF-7 human breast cancer cells and for inhibitory effects on tubulin polymerization and the binding of [^{3}H]colchicine to tubulin, in comparison with the potent colchicine site agent CA-4 [20]. Overall, they had minimal cytotoxic activity and relatively weak effects on colchicine binding, but all the compounds had significant activity as inhibitors of tubulin assembly.

The disulfides are compared with CA-4 in the data presented in Table 2. No disulfide had activity in inhibiting the growth of MCF-7 cells even at 10 μM, as compared with an IC_{50} of 39 nM obtained with CA-4. All the compounds, however, had activity as inhibitors of tubulin assembly, sometimes approaching that of CA-4. This result resembles those previously described with halogenated (Br, Cl and F) analogues of CA-4 [1].

The most active assembly inhibitors among the diaryl disulfides were **2a**, **2c**, **2d** and **2g**, with IC_{50} values ranging from 1.2 (2.2-fold that of CA-4) to 2.4 μM (4.4-fold that of CA-4). Despite the strong inhibition of assembly observed with these diaryl disulfides, none of them was a strong inhibitor of [^{3}H]colchicine binding at 5 μM. Therefore, they were also examined as inhibitors at a 50 μM concentration, and > 30% inhibition was observed with three of these compounds (Table 2). Compound **2a** was the best inhibitor of colchicine binding (53%), while the best inhibitor of tubulin assembly, compound **2g**, inhibited colchicine binding by 34%.

Considering only the four most active inhibitors of assembly, three had the halide substituent in the *ortho* position, with the *o*-bromo (**2g**) the most active and the *o*-fluoro (**2a**) the least active. The third of the best assembly inhibitors was the *p*-fluoro compound **2c**.

It is important to note that the sulfides are symmetrical compounds, whereas the diaryl thiosulfonates are not, although in the latter the halide substituents on the two phenyl rings are also identical. The data obtained with the thiosulfonates in presented in Table 2. Again, most of the compounds did not inhibit MCF-7 growth at 10 μM, but **3c** yielded an IC_{50} of 7.5 μM and **3f** of 10 μM. It is important to point out that the tubulin assembly experiments with the thiosulfonates were performed with a different tubulin preparation than that used with the thioethers, and the values obtained should be compared with those obtained for CA-4 in each experimental series. Using the same criterion with the diaryl thiosulfonates as with the thioethers for activity as inhibitors of assembly, two diaryl thiosulfonates (**3c** and **3g**) were as active as CA-4, while 4 other compounds (**3b**, **3e, 3h** and **3i**) were one-half to one-fourth as active as CA-4. The remaining three compounds were 15–20% as active as CA-4 as inhibitors of tubulin assembly. What immediately stands out is that three of the least active compounds (**3d**, **3e** and **3f**) all bear chloro substituents on the phenyl rings (Table 3). Compound **3c**, with *p*-fluoro substituents, was the best of the diaryl thiosulfonate compounds for all three biological parameters examined, with activity slightly better than CA-4 as an inhibitor of tubulin assembly. Compound **3g** was as active as CA-4 as an inhibitor of tubulin assembly, and it was reasonably active as an inhibitor of colchicine binding.

Compound **3c** is of note because, among the diaryl thiosulfonates, it had the best activity in all three biological parameters studied, and fluorinated stilbenes showed broad-spectrum anticancer activity in different cell lines [1, 21], especially against the MCF-7 cell line [1]. Compound **3f** was the least active of the diaryl thiosulfonates in the assembly assay and weakly cytotoxic, with an IC_{50} of 10 μM against the MCF-7 cells. Aryl chlorine compounds in a different chemotype showed potent cytotoxic activity in the MCF-7 cell line [22]. Other disulfides [23–25] and thiosulfonates [25] have shown cytotoxic activity, but a comparison between these compounds reveals that thiosulfonates are the more active [25].

Perhaps the most striking feature in our results is that the diaryl thiosulfonates were much better inhibitors than the thioethers of [^{3}H]colchicine binding to tubulin, granted that this activity was still very weak compared with that of CA-4. The colchicine inhibition effect was most prominent with the compounds at 50 μM, where all except **3d** inhibited binding of the radiolabeled ligand by over 50%.

Molecular modeling

A bridge containing at least one sulfur atom seems to play an important role in binding to the colchicine site of tubulin, probably due to its atomic volume and electronegativity, aided by both steric and hydrophobic factors [23]. However, the differences between the results of the biological assays of diaryl disulfide and diaryl thiosulfonate derivatives indicate that the chemical features of the bridge have a direct influence on the biological potential of the compounds

Our modeling studies did show that the diaryl disulfides and diaryl thiosulfonates had noticeable differences in their binding mode positions and in the intermolecular interactions made with the amino acid residues of the active site. The interaction of compound **3c**, the

most active of the diaryl thiosulfonates, bound in the colchicine site on β-tubulin, is shown in Figure 2A. The thiosulfonate moiety made hydrophobic interactions with CYS 241 and ALA 250 and a hydrogen bond with ASN 258 (Figure 2A). These observed intermolecular interactions are consistent with those already reported for this type of compound at the colchicine site [26]. In contrast, considering the same amino acid residues, the most active disulfide compound **2g** only made hydrophobic interactions with ALA 354, CYS 241 and ALA 250 (Figure 2B).

The absence of the hydrogen bond with ASN 258 made the disulfide molecule more flexible at the active site, and the bridge had a different final conformation in comparison with CA-4. Despite the similarity of the three compounds, the overlay after energy minimization demonstrated that CA-4 and the diaryl thiosulfonate derivatives have a more promising 3D similarity in their final conformations (Figure 3). As a result, the binding position of these two compounds is very similar and, consequently, so are most of the intermolecular interactions. It correlates well with the better results observed in the bioassays with the thiosulfonates.

Considering that these compounds were conceived as analogues of CA-4, we should note that typically analogues of CA-4 with the greatest activity have two phenyl rings with asymmetric substituents, and this asymmetry was an important feature for potent antitubulin activity [27, 28]. In addition, a number of biphenyls, modeled on colchicine, were evaluated for antitubulin activity, and these were generally inactive, although the substituents on the two phenyl rings were asymmetric [29]. Overall, these results point to the importance of a rigid bridge between the two phenyl rings, as found in CA-4 or with polar groups capable of making hydrogen bonds, as is the case with the thiosulfonates.

Conclusion

In summary, both synthetic protocols are versatile systems for one-pot synthesis of diaryl disulfides and diaryl thiosulfonates from thiols. The fundamental advantages include efficiency and selectivity, determined by the specific reaction conditions. The thiosulfonates seem more promising than the disulfides as leads for compounds that will bind in the colchicine site of tubulin. Eight of nine thiosulfonates, but only four of nine disulfides, were at least 20% as active as CA-4 as inhibitors of tubulin assembly, and eight of nine thiosulfonates were more potent than all the disulfides as inhibitors of colchicine binding to tubulin. Our molecular models of **2g** and **3c** bound in the colchicine site were consistent with these findings, in that the thiosulfonate **3c** had demonstrated a higher number of intermolecular interactions in comparison with disulfide **2g**. This could be rationalized in that the bridge interaction of **3c** through a hydrogen bond with tubulin imparted rigidity to the bound ligand, while such an interaction could not occur with **2g**. Our next step will be to extend our synthetic work to prepare diaryl sulfonates with asymmetrically substituted phenyl rings to better mimic the asymmetric substituents on the phenyl rings of CA-4.

Experimental

Chemistry

All reagents were analytical grade and used without further purification. TLC was performed on Merck 60 F_{254} precoated silica plates, and spots were detected by UV light and a solution of sulfur vanillin [0.5 g vanillin in 100 mL sulfuric acid/methanol (40:10)]. Purification of products was carried out by column chromatography using silica gel 60 (0.035–0.075 mm) and preparative thin-layer chromatography on silica gel 60 F254 (0.063–0.2 mm). The solvents employed in the reactions and silica gel column chromatography were purified and dried according to procedures found in the literature [30]. All melting points were determined using a Uniscience of Brazil model 498 instrument.

IR spectra were recorded on KBr pellets or thin-film using Perkin-Elmer 683 FTIR and FTIR MB100 Boomen spectrometers and reported as wave lengths (cm^{-1}). All samples submitted for ^{1}H and ^{13}C NMR spectroscopy were dissolved in $CDCl_3$, which was also the internal reference. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were measured with a Bruker Avance DPX-300 spectrometer with $CDCl_3$ as the solvent. Chemical shifts are reported in delta (δ) units, parts per million (ppm) relative to TMS (δ = 0.0) or the residual $CHCl_3$ (δ = 7.24) used as an internal reference standard. The ^{1}H NMR spectra are reported as follows: ppm (multiplicity, coupling constant *J*/Hz, number of protons). Multiplicity is abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), dq (doublet of quartets), dt (doublet of triplets), t (triplet), q (quartet), m (multiplet), td (triplet of doublets). Coupling constants (*J*) are quoted in Hertz and recorded to the nearest 0.1 Hz. Low-resolution mass spectra were run on a Shimadzu GCMS-QP2010 Plus, working in ionization mode by electronic impact (EI) at 70 eV, where some samples were introduced through a direct exposition probe. High-resolution mass spectrometry (HRMS) was acquired on a Micromass Autospec working in ionization mode by EI at 70 eV and on a MicroTOF Bruker Daltonics instrument and analyzed by electrospray ionization (ESI). The structures assigned to the compounds were thus confirmed by the spectral data and fit with the MS data.

General Procedure using $AgNO_3$ / $BF_3 \cdot OEt_2$/ ACN (dry) (System A)

7 mmol of halogenated thiol was dissolved in anhydrous ACN (23 mL). Then, 7.7 mmol of $AgNO_3$ and 0.8 mL of $BF_3O(Et)_2$ were added dropwise to the reaction mixture. The mixture was stirred and monitored at intervals by TLC chromatography (hexane / EtOAc 9:1). After 24 h, the reaction was stopped and treated with ice. After extraction with ethyl acetate, the organic layer was washed with distilled water and brine and dried over $MgSO_4$, followed by filtration under reduced pressure. The products were purified by silica gel (GF DE 500 μm, UNIPLAT) chromatography using hexane/EtOAc (9:1) as the eluent.

General Procedure using $Al(H_2PO_4)_3$ / HNO_3 /ACN (dry) (System B)

32 mmol of halogenated thiol was dissolved in anhydrous ACN. 0.16 mmol of aluminium dihydrogen phosphate (freshly prepared) and concentrated HNO_3 (3 mL) were added to this solution, which was stirred at room temperature (r.t.) and monitored by TLC for 24 h, when it was observed that the consumption of the starting material was complete. The

method was optimized by increasing the temperature to 85 °C, leading to faster formation of the thiosulfonate products with high yields. The workup was performed by neutralizing the mixture by careful addition of a sodium bicarbonate solution. The mixture was extracted using ethyl acetate. The organic layer was washed with distilled water and brine and dried over $MgSO_4$, followed by filtration under reduced pressure. The products were purified by silica gel (GF DE 500 μm, UNIPLAT) chromatography using hexane/EtOAc (9:1) as the eluent.

Disulfides

1-Fluoro-2-[(2-fluorophenyl)disulfanyl]benzene (2a)—Light yellow oil, (**A** (r.t.) 74.3%, **B** (r.t.) 72.0%, **B** (heat) 6.2% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3071, 1593, 1574, 1470, 1447, 1260, 1122, 821, 752. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02–7.13 (m, 4H), 7.22–7.29 (m, 2H), 7.61 (td, J = 7.7 Hz, J = 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 115.5–115.8 (d, $^2J_{C\text{-}F}$ = 21.5 Hz (C-H)), 123.3–123.5 (d, $^2J_{C\text{-}F}$ = 17.1 Hz (C-S)), 124.6–124.7 (d, $^3J_{C\text{-}F}$ = 3.6 Hz (C-H)), 129.6–129.7 (d, $^3J_{C\text{-}F}$ = 7.6 Hz (C-H)), 131.1 (s, (C-H)), 158.8–162.0 (d, $^1J_{C\text{-}F}$ = 245.0 Hz (C-F)); MS (EI) *m/z* (%): 254.05 [M+] (71.79), 190.05 (15.67), 127.05 (100.00), 83 (68.64), 57.00 (16.60).

1-Fluoro-3-[(3-fluorophenyl)disulfanyl]benzene (2b)—Light yellow oil, (**A** (r.t.) 54.0%, **B** (r.t.) 61.5%, **B** (heat) 16.5% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3062, 1596, 1579, 1472, 1429, 1264, 1215, 876, 774, 675. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93–6.98 (m, 2H), 7.27–7.3 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 113.7–114.0 (d, $^2J_{C\text{-}F}$ = 24.6 Hz (C-H)), 114.0–114.3 (d, $^2J_{C\text{-}F}$ = 21.7 Hz (C-H)), 122.5–122.5 (d, $^4J_{C\text{-}F}$ = 2.8 Hz (C-H)), 130.3–130.4 (d, $^3J_{C\text{-}F}$ = 8.6 Hz (C-H)), 138.7–138.8 (d, $^3J_{C\text{-}F}$ = 6.8 Hz (C-S)), 161.3–164.6 (d, $^1J_{C\text{-}F}$ = 247.9 Hz (C-F)); MS (EI) *m*/z (%): 254.00 [M+] (76.46), 221.00 (21.56), 190.05 (23.15), 127.00 (100), 83.00 (88.73).

1-Fluoro-4-[(4-fluorophenyl)disulfanyl]benzene (2c)—White powder, (**A** (r.t.) 83.0%, **B** (r.t.) 78.3% yield); m.p. 67–69 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3069, 1589, 1488, 1229, 1155, 824, 621. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.99 (t, J = 8.4, 4H), 7.43 (q, J = 5.1 Hz, J = 3.5 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 116.1–116.4 (d, $^2J_{C\text{-}F}$ = 22.2 Hz (C-H)), 131.2–131.3 (d, $^3J_{C\text{-}F}$ = 7.9 Hz (C-H)), 132.1–132.2 (d, $^4J_{C\text{-}F}$ = 3.8 Hz (C-S)), 160.9–164.2 (d, $^1J_{C\text{-}F}$ = 246.7 Hz (C-F)); MS (EI) *m/z* (%): 254.00 [M+] (88.30), 190.00 (17.67), 127.00 (100), 83.00 (62.11).

1-Chloro-2-[(2-chlorophenyl)disulfanyl]benzene (2d)—White powder, (**A** (r.t.) 63.0%, **B** (r.t.) 69.9% yield); m.p. 73–75 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3056, 1574, 1449, 1432, 1029, 747, 722, 660. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.11–7.23 (m, 4H), 7.35 (dd, J = 7.6 Hz, J = 1.4 Hz, 2H), 7.54 (dd, J = 7.9 Hz, J = 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 127.2 (C-H), 127.6 (C-H), 127.8 (C-H), 129.7 (C-H), 131.9 (C-S), 134.4 (C-Cl); MS (EI) *m/z* (%): 285.90 [M+] (60.24), 287.90 [M+2] (43.12), 289.90 [M+4] (10.16), 215.95 (21.77), 217.90 (21.65), 221.90 (13.47), 142.95 (82.24), 144.90 (30.59), 108.00 (100.00).

1-Chloro-3-[(3-chlorophenyl)disulfanyl]benzene (2e)—Light yellow oil, (**B** (heat) 18.5% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3053, 1574, 1460, 1406, 1116, 1072, 1072, 867,

772, 675. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19–7.21 (m, 4H), 7.32–7.36 (m, 2H), 7.48 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 125.2 (C-H), 126.8 (C-H), 127.4 (C-H), 130.1 (C-H), 135.0 (C-Cl), 138.3 (C-S); MS (EI) *m*/*z* (%): 285.95 [M+] (80.55), 287.95 [M+2] (58.93), 289.95 [M+4] (13.85), 218.00 (33.88), 222.00 (27.00), 224.00 (17.37), 143.00 (84.11), 144.95 (31.30), 108.00 (100.00).

1-Chloro-4-[(4-chlorophenyl)disulfanyl]benzene (2f)—White powder, (**A** (r.t.) 70.2%, **B** (r.t.) 75.0% yield); m.p. 69–70 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3078, 1896, 1473, 1396, 1094, 1010, 816, 741. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 (d, *J* = 8,6 Hz, 4H), 7.38 (d, *J* = 8.5 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 129.2 (C-H), 133.5 (C-H), 135.1 (C-Cl), 137.6 (C-S); MS (EI) *m*/*z* (%): 285.90 [M+] (39.49), 287.90 [M+2] (28.23), 289.90 [M+4] (6.62), 142.95 (100.00), 144.95 (37.24), 108.00 (64.00), 109,00 (11.90).

1-Bromo-2-[(2-bromophenyl)disulfanyl]benzene (2g)—White powder, (**A** (r.t.) 47.6%, **B** (r.t.) 35.8%, **B** (heat) 9.3% yield); m.p. 86–89 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3052, 1444, 1427, 1016, 742, 704, 650. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.06 (td, *J* = 7.9 Hz, *J* = 1.3 Hz, 2H), 7.24 (td, *J* = 7.7 Hz, *J* = 1.8 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 121.0 (C-Br), 126.9 (C-H), 127.9 (C-H), 128.2 (C-H), 132.9 (C-H), 136.1 (C-S); MS (EI) *m*/*z* (%): 373.75 [M+] (14.57), 375.75 [M+2] (29.97), 377.75 [M+4] (16.38), 215.90 (52.20), 186.85 (10.57), 188.85 (11.03), 108.00 (100.00).

1-Bromo-3-[(3-bromophenyl)disulfanyl]benzene (2h)—Dark yellow oil, (**A** (r.t.) 59.3%, **B** (r.t.) 44.1%, **B** (heat) 10.1% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3051, 1572, 1561, 1456, 1399, 1066, 771, 743, 674. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.14 (t, *J* = 7.9 Hz, 2H), 7.33–7.40 (m, 4H), 7.62 (d, *J* = 1.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 123.1 (C-Br), 125.7 (C-H), 129.8 (C-H), 130.4 (2C-H), 138.5 (C-S); MS (EI) *m*/*z* (%): 373.75 [M+] (21.00), 375.75 [M+2] (41.19), 377.75 [M+4] (23.11), 215.95 (16.29), 186.85 (14.30), 188.85 (14.81), 108.00 (100.00).

1-Bromo-4-[(4-bromophenyl)disulfanyl]benzene (2i)—White powder, (**A** (r.t.) 76.6%, **B** (r.t.) 62.7% yield); m.p. 84–85 ºC. FT-IR (KBr, cm^{-1}) ν_{max}: 3073, 1468, 1384, 1080, 1006, 826, 812, 723.^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.31 (d, *J* = 8.59 Hz, 4H), 7.41 (d, *J* = 8.3 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 121.5 (C-Br), 129.4 (C-H), 132.2 (C-H), 135.7 (C-S); MS (EI) *m*/*z* (%): 373.80 [M+] (23.72), 375.80 [M+2] (48.89), 377.80 [M+4] (26.09), 186.90 (40.59), 188.90 (41.60), 108.00 (100.00).

Thiosulfonates

1-Fluoro-2-[(2-fluorobenzenesulfonyl)sulfanyl]benzene (3a)—Light yellow oil, (**A** (r.t.) 10.2%, **B** (r.t.) 12.4%, **B** (heat) 87.0% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3071, 1574, 1470. 1447, 1260, 1222, 1156, 821, 752, 671, 549. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (td, *J* = 8.5 Hz, *J* = 1.0 Hz, 1H), 7.12 (td, *J* = 7.5 Hz, *J* = 1.1 Hz, 1H), 7.15 (td, *J* = 6.4 Hz, *J* = 1.1 Hz, 1H), 7.23 (td, *J* = 8.5 Hz, *J* = 1.0 Hz, 1H), 7.42–7.52 (m, 3H), 7.57–7.64 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 114.6–114.9 (d, $^{2}J_{C\text{-}F}$ = 18.9 Hz (C-S)), 116.2–116.5 (d, $^{2}J_{C\text{-}F}$ = 22.8 Hz (C-H)), 117.5–117.8 (d, $^{2}J_{C\text{-}F}$ = 21.1 Hz (C-H)), 123.8–123.9 (d, $^{3}J_{C\text{-}F}$ = 3.4 Hz (C-H)), 125.0–125.1 (d, $^{3}J_{C\text{-}F}$ = 4.0 Hz (C-H)), 130.2 (s, (C-H)), 131.1–131.3 (d,

$^{2}J_{C\text{-}F}$ = 12.0 Hz (C-S)), 134.5–134.6 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz (C-H)), 136.3–136.4 (d, $^{3}J_{C\text{-}F}$ = 8.5 Hz (C-H)), 139.1 (s, (C-H)), 157.2–160.6 (d, $^{1}J_{C\text{-}F}$ = 258.8 (C-F)), 161.2–164.6 (d, $^{1}J_{C\text{-}F}$ = 251.8 Hz (C-F)); HRMS (ESI) $C_{12}H_8F_2O_2S_2$ [M+] *m/z* (%) calc. 285.9933, found 285.9933.

1-Fluoro-3-[(3-fluorobenzenesulfonyl)sulfanyl]benzene (3b)—Yellow oil, (**B** (r.t.) 20.3%, **B** (heat) 63.0% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 3062, 1596, 1582, 1472, 1429, 1264, 1215, 876, 774, 675, 520. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.04–7.16 (m, 3H), 7.21–7.31 (m, 4H), 7.35–7.41 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 114.6–114.9 (d, $^{2}J_{C}$-F = 24.6 Hz (C-H)), 118.8–119.1 (d, $^{2}J_{C\text{-}F}$ = 21.1 Hz (C-H)), 121.0–121.3 (d, $^{2}J_{C\text{-}F}$ = 21.1 Hz (C-H)), 123.0–123.3 (d, $^{2}J_{C\text{-}F}$ = 22.3 Hz (C-H)), 123.3 (s, (C-H)), 128.9–129.0 (d, $^{3}J_{C\text{-}F}$ = 8.3 Hz (C-S)), 130.6–130.7 (d, $^{3}J_{C\text{-}F}$ = 8.5 Hz (C-H)), 130.8 (s, (C-H)),132.1–132.2 (d, $^{3}J_{C\text{-}F}$ = 3.0 Hz (C-H)), 144.3–144.4 (d, $^{3}J_{C\text{-}F}$ = 6.4 Hz (C-S)), 160.3–163.7 (d, $^{1}J_{C\text{-}F}$ = 251.4 Hz (C-F)), 160.6–163.9 (d, $^{1}J_{C\text{-}F}$ = 250.1 Hz (C-F)); HRMS (ESI) $C_{12}H_8F_2O_2S_2$ [M+] *m/z* (%), calc. 285.9933, found 285.9934.

1-Fluoro-4-[(4-fluorobenzenesulfonyl)sulfanyl]benzene (3c)—White powder, (**A** (r.t.) 13.7%, **B** (r.t.) 9.0%, **B** (heat) 95.0% yield); m.p. 67–69 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3101, 3070, 1585, 1489, 1335, 1238, 1146, 1072, 833, 660, 586, 517. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.00–7.12 (m, 4H), 7.34 (q, *J* = 8.6 Hz, *J* = 1.7 Hz, 2H), 7.56 (q, *J* = 8.8 Hz, *J* = 1.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 116.0–116.3 (d, $^{2}J_{C\text{-}F}$ = 22.3 Hz, (C-H)), 116.8–117.1 (d, $^{2}J_{C\text{-}F}$ = 22,0 Hz, (C-H)), 123.2 (d, $^{4}J_{C\text{-}F}$ = 2.9 Hz (C-S)), 130.4–130.5 (d, $^{3}J_{C\text{-}F}$ = 9.2 Hz, (C-H)), 138.8–138.9 (d, $^{3}J_{C\text{-}F}$ = 9.2 Hz, (C-H)), 163.2–166.5 (d, $^{1}J_{C\text{-}F}$ = 254.4 Hz, (C-F)), 163.9–167.3 (d, $^{1}J_{C\text{-}F}$ = 255.9 Hz, (C-F)); HRMS (ESI) $C_{12}H_8F_2O_2S_2$ [M+] *m/z* (%), calc. 285.9933, found 285.9927.

1-Chloro-2-[(2-chlorobenzenesulfonyl)sulfanyl]benzene (3d)—White powder, (**A** (r.t.) 15.5%, **B** (r.t.) 11.5, **B** (heat) 83.8% yield); m.p. 81–83 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3055, 1821, 1783, 1597, 1573, 1564, 1477, 1431, 1029, 747, 723, 660. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19–7.40 (m, 4H), 7.48–7.58 (m, 3H), 7.63 (dd, *J* = 7.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 126.6 (C-H), 126.8 (C-Cl), 127.6 (C-H), 130.2 (C-H), 131.0 (C-H), 132.4 (C-H), 133.0 (C-Cl), 133.1 (C-H), 134.7 (C-H), 139.9 (C-H), 140.3(C-S), 140.4 (C-S); MS (EI) *m/z* (%): 317.85 [M+] (21.93), 319.85 [M+2] (16.20), 321.85 [M+4] (3.69), 174.95 (34.49), 176.90 (12.33), 158.95 (100.0), 160.90 (36.48), 143,95 (30.87), 107.95 (55.79), 110.95 (41.25), 112.95 (13.66).

1-Chloro-3-[(3-chlorobenzenesulfonyl)sulfanyl]benzene (3e)—Yellow oil, (**B** (heat) 67.3% yield). FT-IR (KBr, cm^{-1}) ν_{max}: 2926, 1573, 1460, 1338, 1151, 1074, 783, 676, 604, 535. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.15–7.29 (m, 3H), 7.31–7.44 (m, 3H), 7.47–7.53 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 125.6 (C-H), 127.6 (C-H), 129.0 (C-Cl), 130.2 (C-H), 130.6 (C-H), 131.9 (C-H), 134.0 (C-H), 134.6 (C-H), 135.1 (C-Cl), 135.3 (C-S), 136.1 (C-H), 144.0 (C-S); MS (EI) *m/z* (%): 317.95 [M+] (18.11), 319.95[M+2] (13.14), 321.90 [M+4] (3.84), 281.05 (9.02), 207.00 (14.50), 175.95 (41.23), 177.00 (16.82), 159.00 (74.25), 160.95 (27.28), 143.0 (34.77), 143.95 (71.66), 146.00 (28.74), 111.00 (100.00), 108.00 (94.99), 75.00 (71.82).

1-Chloro-4-[(4-chlorobenzenesulfonyl)sulfanyl]benzene (3f)—White powder, (**A** (r.t.) 12.0%, **B** (r.t.) 13.8%, **B** (heat) 92.0% yield); m.p. 134–135 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 2923, 1573, 1450, 1330, 1149, 1033, 756, 597. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.27–7.31 (m, 4H), 7.41 (dt, *J* = 8.9 Hz, *J* = 2.1 Hz, 2H), 7.50 (dt, *J* = 9.1 Hz, *J* = 1.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 125.9 (C-Cl), 128.9 (C-H), 129.2 (C-H), 129.9 (C-H), 137.6 (C-H), 138.5 (C-S), 140.5 (C-Cl), 141.2 (C-S); HRMS (ESI) $C_{12}H_8Cl_2O_2S_2$ [M+] *m/z* (%), calc. 317.9342, found 317.9342.

1-Bromo-2-[(2-bromobenzenesulfonyl)sulfanyl]benzene (3g)—White powder, (**A** (r.t.) 9.2%, **B** (heat) 80.6% yield); m.p. 128–131 ºC. FT-IR (KBr, cm^{-1}) ν_{max}: 3082, 1569, 1446, 1326, 1149, 1018, 752, 594, 540. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25–7.29 (m, 2H), 7.33 (td, *J* = 4.6 Hz, *J* = 0.9 Hz, 1H), 7.41 (td, *J* = 4.6 Hz, *J* = 1.0 Hz, 1H), 7.52 (dd, *J* = 4.7 Hz, *J* = 0.8 Hz, 1H), 7.56 (dd, *J* = 4.8 Hz, *J* = 1.0 Hz, 1H), 7.68 (dd, *J* = 4.7 Hz, *J* = 1.1 Hz, 1H), 7.78 (dd, *J* = 4.7 Hz, *J* = 0.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 121.3 (C-Br), 127.2 (C-H), 128.3 (C-H), 129.1 (C-Br), 131.2 (C-S), 131.3 (C-H), 133.0 (C-H), 133.6 (C-H), 134.6 (C-H), 136.0 (C-H), 140.1 (C-H), 142.0 (C-S); HRMS (ESI) $C_{12}H_8Br_2O_2S_2$ [M+] *m/z* (%), calc. 405.8332, found 405.8334.

1-Bromo-3-[(3-bromobenzenesulfonyl)sulfanyl]benzene (3h)—White powder, (**B** (heat) 84.4% yield); m.p. 91–93 ºC. FT-IR (KBr, cm^{-1}) ν_{max}: 3086, 3070, 1570, 1558, 1458, 1327, 1146, 787, 756, 671, 602, 528. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.26 (t, *J* = 7.8 Hz, 1H), 7.33 (td, *J* = 7.8 Hz, *J* = 1.8 Hz, 2H), 7.44 (t, *J* = 1.8 Hz, 1H), 7.49 (dq, *J* = 7.9 Hz, *J* = 0.9 Hz, 1H), 7.63 (dq, *J* = 7.9 Hz, *J+* = 1.2 Hz, 2H), 7.72 (dq, *J* = 78.0 Hz, *J* = 1.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 122.9 (2C-Br), 126.0 (C-H), 129.3 (C-S), 130.4 (C-H), 130.5 (C-H), 130.9 (C-H), 134.8 (C-H), 135.1 (C-H), 136.9 (C-H), 138.9 (C-H), 144.1 (C-S); HRMS (ESI) $C_{12}H_8Br_2O_2S_2$ [M+] *m/z* (%), calc. 405.8332, found 405.8326.

1-Bromo-4-[(4-bromobenzenesulfonyl)sulfanyl]benzene (3i)—White powder, (**A** (r.t.) 17.0%, **B** (heat) 93.0% yield); m.p. 153–154 °C. FT-IR (KBr, cm^{-1}) ν_{max}: 3086, 2924, 1566, 1331, 1146, 1068, 1007, 822, 741, 598, 552. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22 (dt, *J* = 9.2 Hz, *J* = 2.2 Hz, 2H), 7.42 (dt, *J* = 9.1 Hz, *J* = 2.6 Hz, 2H), 7.50 (dt, *J* = 9.6 Hz, *J* = 2.4 Hz, 2H), 7.58 (dt, *J* = 9.1 Hz, *J* = 2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 126.6 (C-Br), 127.0 (C-Br), 129.0 (C-H), 129.2 (C-S), 132.3 (C-H), 132.9 (C-H), 137.8 (C-H), 141.9 (C-S); HRMS (ESI) $C_{12}H_8Br_2O_2S_2$ [M+] *m/z* (%), calc. 405.8332, found 405.8314.

Bioassays

The tubulin assembly [31] and inhibition of colchicine binding to tubulin [32] assays were performed as described before. In the assembly assay, the tubulin concentration was 10 μM, and the parameter measured was the extent of assembly after 20 min at 30 °C. In the colchicine binding assay, the tubulin concentration was 1.0 μM, the [^{3}H]colchicine concentration was 5.0 μM, and the inhibitor concentration was 5 or 50 μM, as indicated. Incubation was for 10 min at 37 °C, a time point chosen because the control reaction is about 40–60% complete. The MCF-7 cytotoxicity assay was performed as described by Monks et al., and protein, stained by sulforhodamine B, was the cell parameter measured [33].

Molecular modeling

The 3D structures of the most active disulfide and thiosulfonate compounds were drawn using the program MarvinSketch 16.9.5 (ChemAxon Ltd.). The structural optimization was made through the PM7 semi-empirical method incorporated in the software MOPAC2016 [34].

To determine the potential binding model assumed by both compounds (**2g** and **3c**), molecular docking simulations were carried out using AutoDockVINA [35]. For the simulations, the protein from PDB ID 5LYJ with CA-4 as the bound ligand (ligand PDB ID: 7BA) was used and the grid box determined was large enough to contain the binding site of the target. Before each simulation, the crystallographic ligand was deleted. The best result obtained by the molecular docking simulation for both compounds was energy minimized with the program GROMAC 5 package [36] and CHARMM force field [37]. The properties of the solvent were determined based on the TIP3P water model, and a cubic box that guarantees a space of 1.2 nm between the protein and box walls was used. All system charges were neutralized with the addition of ions at physiological concentration (0.15 μM). The algorithm steepest descent minimization and conjugated gradients were used with a maximum force of 10 KJ/mol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Development high yield and versatile one-pot synthesis for diaryl disulfides and diaryl thiosulfonates;
- The thiosulfonates have demonstrated to be promising as a lead compound for the development of new inhibitors of colchicine site of tubulin.
- Molecular modeling studies were consistent with the *in vitro* results

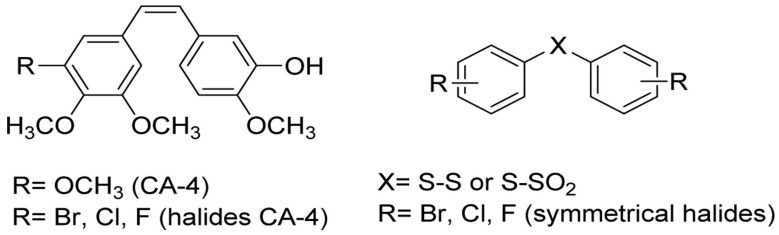


Figure 1.
Structures of CA-4, halide analogues of CA-4 and diaryl disulfides and diaryl thiosulfonates

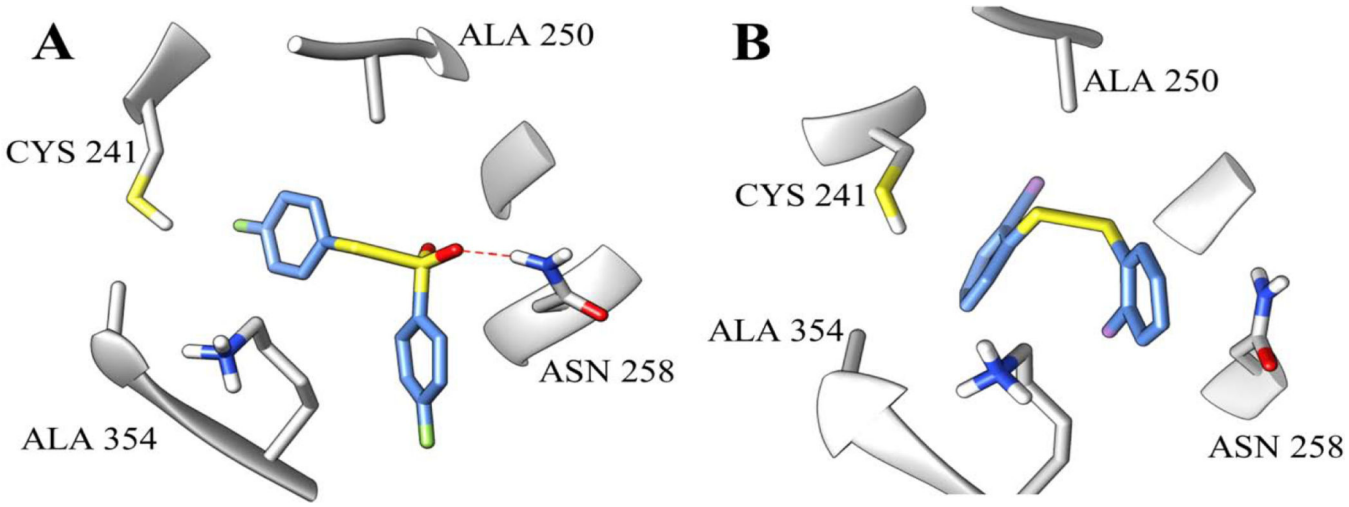


Figure 2.

Binding modes of the diaryl thiosulfonate **3c** (A) and the diaryl disulfide **2g** (B) at the colchicine site in β-tubulin (PDB ID: 5LYJ).

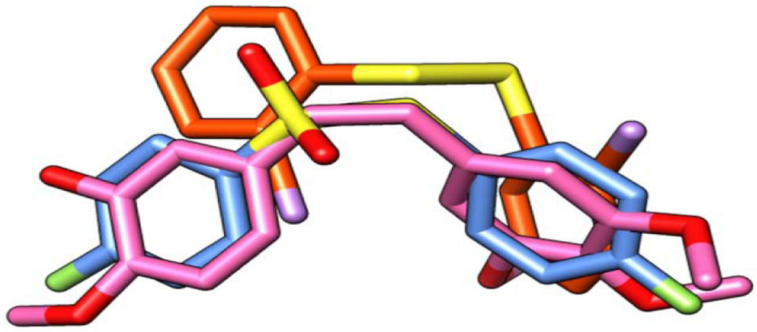
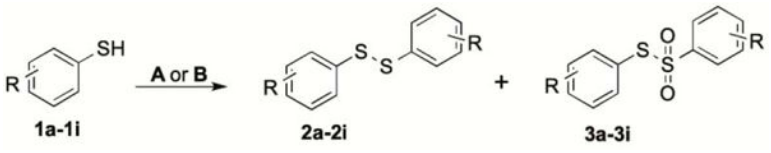


Figure 3.
Overlay of CA-4 (pink), diaryl thiosulfonate **3c** (blue) and diaryl disulfide **2g** (orange)

Table 1.

Reaction yields (in parentheses) using system **A**[a] or **B**[b]



Entry	Thiol (R)	Yield (%)					
		System A (r.t.)		System B (r.t.)		System B (heat)	
		Diaryl disulfide	Diaryl thiosulfonate	Diaryl disulfide	Diaryl thiosulfonate	Diaryl disulfide	Diaryl thiosulfonate
1	**1a** (*o*-F)	**2a** (74.3)	**3a** (10.2)	**2a** (72.0)	**3a** (12.4)	**2a** (6.2)	**3a** (87.0)
2	**1b** (*m*-F)	**2b** (54.0)	-	**2b** (61.5)	**3b**[c] (20.3)	**2b** (16.5)	**3b**[c] (63.0)
3	**1c** (*p*-F)	**2c** (83.0)	**3c** (13.7)	**2c** (78.3)	**3c** (9.0)	**2c** (0.0)	**3c** (95.0)
4	**1d** (*o*-Cl)	**2d** (63.2)	**3d** (15.5)	**2d** (69.9)	**3d** (11.5)	**2d** (0.0)	**3d** (83.8)
5	**1e** (*m*-Cl)	-	-	-	-	**2e** (18.5)	**3e** (67.3)
6	**1f** (*p*-Cl)	**2f** (70.2)	**3f** (12.0)	**2f** (75.0)	**3f** (13.8)	**2f** (0.0)	**3f** (92.0)
7	**1g** (*o*-Br)	**2g** (47.6)	**3g** (9.2)	**2g** (35.8)	**3g** (0.0)	**2g** (9.3)	**3g**[c] (80.6)
8	**1h** (*m*-Br)	**2h** (59.3)	**3h** (0.0)	**2h** (44.1)	**3h** (0.0)	**2h** (10.1)	**3h** (84.4)
9	**1i** (*p*-Br)	**2i** (76.6)	**3i** (0.0)	**2i** (62.7)	**3i** (17.0)	**2i** (0.0)	**3i** (93.0)

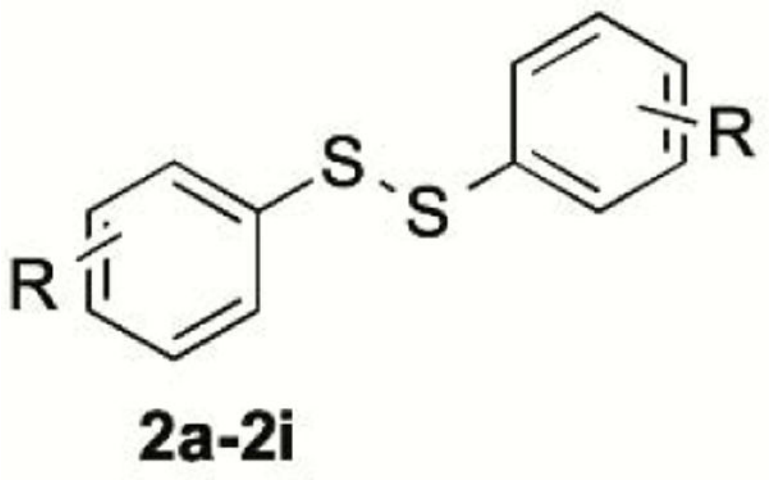
[a] $AgNO_3/BF_3{\cdot}OEt_2$, ACN (dry)

[b] $Al(H_2PO_4)_3$-HNO_3, ACN (dry)

(–) reaction not performed

Table 2:

Bioassays with diaryl disulfides

 **2a-2i**	IC_{50} µM ± SD			
	Inhibition of tubulin assembly[a] (relative to CSA4)	**Inhibition of MCF-7 cell growth[b]**	**Inhibition of colchicine binding[c] % Inhibition ± SD**	
R = *ortho (o), meta (m), para (p)* - F, Cl, Br			**5 µM inhibitor**	**50 µM inhibitor**
CA-4	0.54 ± 0.06	0.039 ± 0.006	98 ± 1	100 ± 0.04
(**2a**) *o*-F	2.4 ± 0.3 (4.4)	> 10	0 ± 4	53 ± 5
(**2b**) *m*-F	4.8 ± 0.1 (8.9)	> 10	1.4 ± 4	12 ± 2
(**2c**) *p*-F	1.9 ± 0.06 (3.5)	> 10	2.6 ± 4	17 ± 4
(**2d**) *o*-Cl	1.4 ± 0.2 (2.6)	> 10	15 ± 5	36 ± 2
(**2e**) *m*-Cl	3.1 ± 0.4 (5.7)	> 10	12 ± 2	18 ± 5
(**2f**) *p*-Cl	4.6 ± 0.7 (8.5)	> 10	9.8 ± 2	0 ± 4
(**2g**) *o*-Br	1.2 ± 0.1 (2.2)	> 10	27 ± 3	34 ± 4
(**2h**) *m*-Br	4.0 ±0.5 (7.4)	> 10	6.6 ± 5	17 ± 4
(**2i**) *p*-Br	5.1 ± 0.5 (9.4)	> 10	11 ± 0.3	0 ± 2

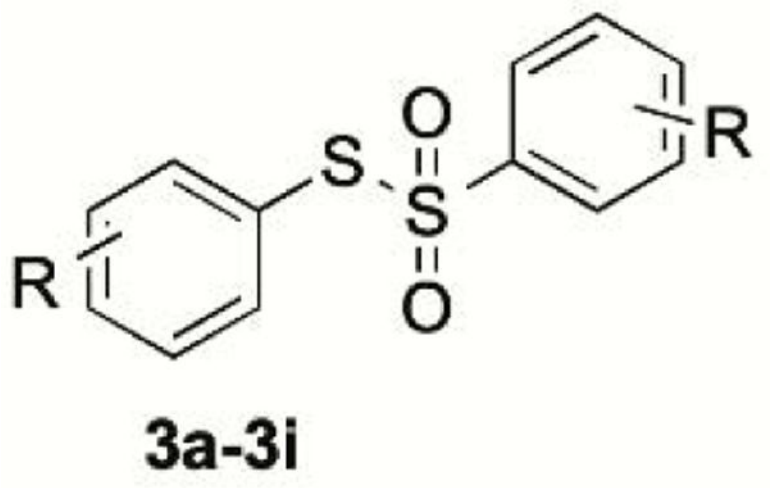
[a] Averages of three independent experiments.

[b] Single determination, except for CA-4.

[c] Averages of two independent experiments.

Table 3:

Bioassays with diaryl thiosulfonates



R = *ortho (o), meta (m), para (p)* - F, Cl, Br	IC$_{50}$ μM ± SD: Inhibition of tubulin assembly[a] (relative to CSA4)	IC$_{50}$ μM ± SD: Inhibition of MCF-7 cell growth[b]	Inhibition of colchicine binding[c] % Inhibition ± SD: 5 μinhibitor	50 μM inhibitor
CA-4	1.3 ± 0.1	0.021 ± 0.004	98 ± 1	100 ± 0.04
(**3a**) *o*-F	5.5 ± 0.1 (4.2)	> 10	0	75 ± 5
(**3b**) *m*-F	3.1 ± 0.2 (2.4)	> 10	8.9 ± 0.9	97 ± 0.1
(**3c**) *p*-F	1.2 ± 0.06 (0.92)	7.5 ± 0[d]	52 ± 5	100 ± 0.3
(**3d**) *o*-Cl	6.3 ± 0.3 (4.8)	> 10	12 ± 2	31 ± 4
(**3e**) *m*-Cl	4.7 ± 0.04 (3.6)	> 10		93 ± 1
(**3f**) *p*-Cl	9.1 ± 0.9 (7.0)	10 ± 0[d]	0	75 ± 2
(**3g**) *o*-Br	1.3 ± 0.06 (1.0)	> 10	18 ± 5	90 ± 0.3
(**3h**) *m*-Br	3.3 ± 0.04 (2.5)	> 10	20 ± 1	99 ± 1
(**3i**) *p*-Br	4.2 ± 0.2 (3.2)	> 10	6.6 ± 4	80 ± 2

[a] Averages of three independent experiments.

[b] Single determination, except for CA-4, **3c** and **3f**.

[c] Averages of two independent experiments.

[d] The same value was obtained twice.