A GENUINELY MULTIDISCIPLINARY JOURNAL

CHEMPLUSCHEM

CENTERING ON CHEMISTRY

Accepted Article

Title: Microwave-Assisted Synthesis of Sulfurated Heterocycles with Herbicidal Activity: Reaction of 2-Alkynylbenzoic Acids with Lawesson's Reagent

Authors: Salvatore V. Giofrè, Raffaella Mancuso, Fabrizio Araniti, Roberto Romeo, Daniela Iannazzo, Maria Rosa Abenavoli, and Bartolo Gabriele

To be cited as: *ChemPlusChem* 10.1002/cplu.201900316

Link to VoR: http://dx.doi.org/10.1002/cplu.201900316

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chempluschem.org

Microwave-Assisted Synthesis of Sulfurated Heterocycles with Herbicidal Activity: Reaction of 2-Alkynylbenzoic Acids with Lawesson's Reagent

Salvatore V. Giofrè,*[a] Raffaella Mancuso,[b] Fabrizio Araniti,*[c] Roberto Romeo,[a] Daniela Iannazzo,[a] Maria Rosa Abenavoli[c] and Bartolo Gabriele*[b]

Abstract: The reactivity of 2-alkynylbenzoic acids toward the Lawesson's reagent (LR) agent under microwave irradiation (300 W, 100 °C, CH_2Cl_2) was assessed. It was found that, depending on reaction conditions, either a dithionation- or a monothionation-cycloisomerization process may take place with formation of important sulfurated heterocycles. In particular, using 1 equiv of the LR for 1 h, dithionation occurred, with formation of benzo[*c*]thiophene-1(3*H*)-thiones or 1*H*-isothiochromene-1-thiones, while with 0.5 equiv of the LR for 10-30 min reaction time, monothionated products were selectively obtained (benzo[*c*]thiophen-1(3*H*)-ones or 1*H*-isothiochromen-1-ones). The regiochemical output of the process strongly depended on the substitution pattern of the starting 2-alkynylbenzoic acid derivatives. These compounds were also assayed as potential herbicides, by assessing their phytotoxic activity on seedling growth and development of the model species *Arabidopsis Thaliana*. All compounds, at different extent, influenced the morpho-physiological parameters monitored; in particular, the Fresh Weight (FW) was significantly affected, with ED_{50} values ranging from 4.81 μM to 63.7 μM.

Introduction

In recent years, global agriculture has been facing the fundamental problem related to the rapid growth of the world population associated with the scarcity of land available for cultivation. To tackle this issue, agricultural production is expected to increase significantly, and this increase can be achieved either by increasing yield or by minimizing productivity loss. In this regard, the agrochemical industry, which produces fertilizers and pesticides, plays a vital role.[1] Indeed, fertilizers help to achieve a higher yield in agricultural production, while pesticides tend to minimize the loss of productivity caused by harmful agents.[2] In particular, pesticides include herbicides, fungicides, insecticides and nematicides.[3]

On the other hand, the intensive and, sometimes, indiscriminate use of these products may represent a major cause of environmental pollution and a potential risk for human health. Concerning in particular herbicides, the widespread expansion of weed resistance towards traditional active principles is becoming an additional and stringent issue. This justifies the continuous effort toward the development of new classes of synthetic molecules, characterized by high biological activity and new mechanisms of action (MOA), in conjunction with safer toxicological and environmental profiles. Among agrochemicals, sulfur-containing compounds are of particular importance because of the biological role of this element. Currently, in fact, more than 30% of agrochemicals contain at least a sulfur atom, particularly in insecticides, herbicides, and fungicides.[4]

In this paper, we report a novel synthetic approach to important classes of sulfurated heterocyclic derivatives, which display a significant and promising herbicidal activity. The synthetic method is based on tandem thionation-heterocyclization reactions, starting from readily available 2-alkynylbenzoic acids, carried out in the presence of the Lawesson's reagent (LR) and under microwave (MW) irradiation.

Results and Discussion

Synthesis of sulfurated heterocycles by thionation-cycloisomerization of 2-alkynylbenzoic acids under MW irradiation

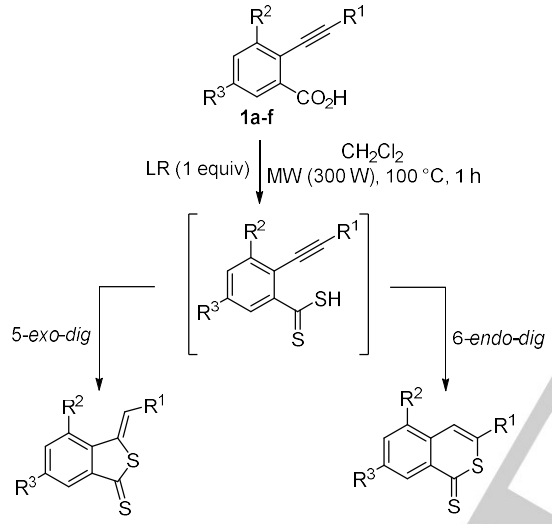
Recently, we communicated the possibility to synthesize either benzo[*c*]thiophene-1(3*H*)-thiones **2** or 1*H*-isothiochromene-1-thiones **3** by dithionation-cycloisomerization of 2-alkynylbenzoic acids **1**, using 1 equiv of the LR as the thionating agent under MW irradiation (at 100 °C and 300 W for 1 h in CH_2Cl_2), as shown in Scheme 1.[5-7] In particular, benzothiophenethiones **2** (from a 5-*exo*-*dig* cyclization mode) were selectively obtained from substrates bearing an aryl group on the triple bond (either unsubstituted or substituted with an electron-withdrawing group (EWG), such as a *p*-fluorine). On the other hand, isothiochromenethiones **3** (from 6-*endo*-*dig* cyclization) were formed from substrates bearing, on the triple bond, an alkyl group or an aryl group substituted with a π-donating group (ERG) (such as a *p*-methoxyl) (Scheme 1).[5] The regiochemical output of the process therefore depended on the electronic nature of the triple bond. In particular, an EWG on the triple

[a] Dr. S. V. Giofrè, Prof. Dr. R. Romeo, Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, Università di Messina, via S.S. Annunziata, 98168 Messina, Italy.
E-mail: sgiofre@unime.it (S.V.G.)
[b] Dr. R. Mancuso, Prof. Dr. B. Gabriele, Laboratory of Industrial and Synthetic Organic Chemistry (LISOC), Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria, via P. Bucci 12/C, 87036 Arcavacata di Rende, CS, Italy.
E-mail: bartolo.gabriele@unical.it (B.G.)
[c] Dr. F. Araniti, Prof. Dr. M. R. Abenavoli, Dipartimento AGRARIA, Università Mediterranea di Reggio Calabria, Reggio Calabria 89124, Italy.
E-mail: fabrizio.araniti@unirc.it (F.A.)

Supporting information for this article is given via a link at the end of the document.

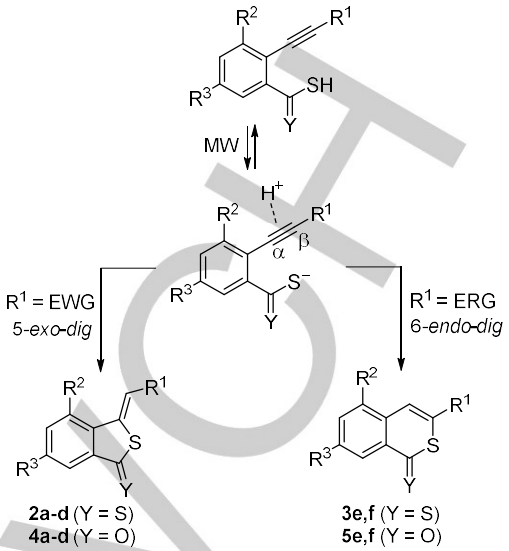
bond favored protonation of the β-carbon of the triple bond, with formation of **2**, while an ERG on the triple bond directed the protonation to the α-carbon, with formation of **3** (Scheme 2, Y = S).[5]

We have now found that, under the above-mentioned conditions (MW irradiation at 300 W, 100°C, in CH_2Cl_2), but using 0.5 equiv of LR with respect to the 2-alkynylbenzoic acid **1** and after a shorter reaction time (10-30 min), it is possible to selectively convert **1a-f** into monothionated products (benzo[*c*]thiophen-1(3*H*)-ones **4a-d** or 1*H*-isothiochromen-1-ones **5e,f**), depending again on the kind of substituent on the triple bond (Scheme 3). These products clearly derive from 5-*exo*-*dig* or 6-*endo*-*dig* cycloisomerization of the corresponding 2-ethynylbenzothioic acids (formed in situ by monothionation of **1**), as shown in Scheme 2 (Y = O).

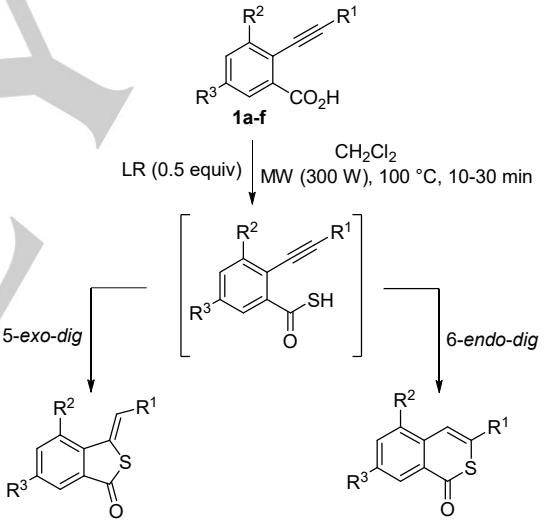


2a (R^1=Ph, R^2=R^3=H): 90%
2b (R^1=Ph, R^2=Me, R^3=H): 91%
2c (R^1=Ph, R^2=H, R^3=Cl): 85%
2d (R^1=*p*-F-C_6H_4, R^2=R^3=H): 88%
3e (R^1=*p*-$MeOC_6H_4$, R^2=R^3=H): 90%
3f (R^1=Pr, R^2=R^3=H): 86%

Scheme 1. Divergent syntheses of benzo[*c*]thiophene-1(3*H*)-thiones **2a-d** or 1*H*-isothiochromene-1-thiones **3e,f** by dithionation-cycloisomerization of 2-alkynylbenzoic acids **1a-f**.[5]



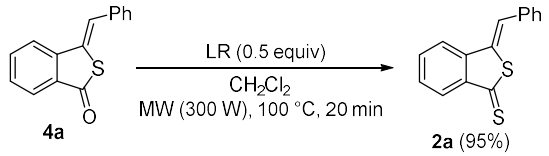
Scheme 2. Divergent pathways for the cycloisomerization of 2-alkynylbenzo(di)thioic acids leading to 5-membered sulfurated heterocycles (**2a-d** or **4a-d**) or 6-membered sulfurated heterocycles (**3e,f** or **5e,f**).



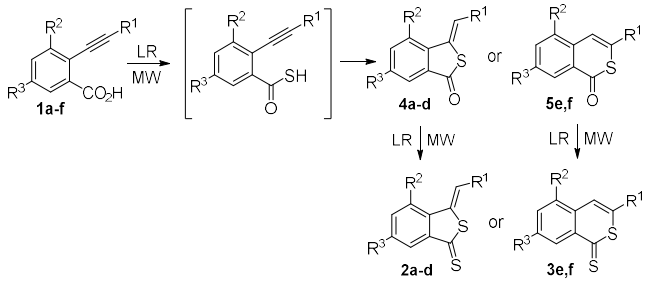
4a (R^1=Ph, R^2=R^3=H): 84%
4b (R^1=Ph, R^2=Me, R^3=H): 84%
4c (R^1=Ph, R^2=H, R^3=Cl): 82%
4d (R^1=*p*-F-C_6H_4, R^2=R^3=H): 84%
5e (R^1=*p*-$MeOC_6H_4$, R^2=R^3=H): 80%
5f (R^1=Pr, R^2=R^3=H): 78%

Scheme 3. Divergent syntheses of benzo[*c*]thiophen-1(3*H*)-ones **4a-d** or 1*H*-isothiochromen-1-ones **5e,f** by thionation-cycloisomerization of 2-alkynylbenzoic acids **1a-f**.

Interestingly, we have also found that dithionated product **2a** can be formed by allowing the corresponding monothionated derivative **4a** to react with 0.5 equiv of the LR for 20 min under the usual conditions (Scheme 4). This suggests that, as an alternative to the mechanism shown in Scheme 2, products **2a-d** and **3e,f** might also ensue from a subsequent thionation of the initially formed monothionated compounds **4a-d** and **5e,f**, respectively (Scheme 5).

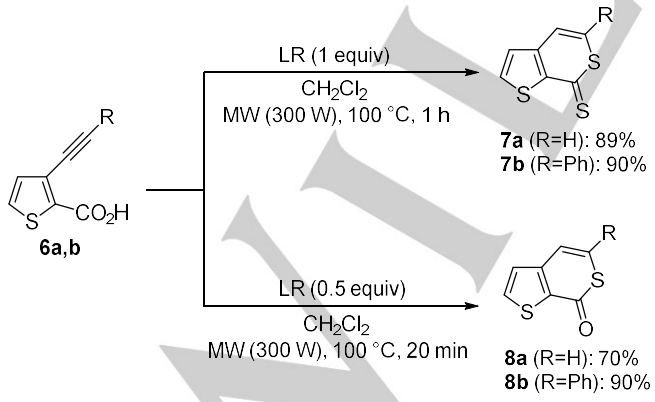


Scheme 4. Thionation of (*Z*)-3-benzylidenebenzo[c]thiophen-1(3*H*)-one **4a** into (Z)-3-benzylidenebenzo[*c*]thiophene-1(3*H*)-thione **2a**.



Scheme 5. Possible alternative routes for the formation of dithionated products from 2-alkynylbenzoic acids **1a-f**: monothionation to give monosulfurated heterocycles **4a-d** or **5e,f** followed by thionation of the latter to give **2a-d** and **3e,f**, respectively.

To expand the scope of our synthetic methodology, we also assessed the reactivity of 3-ethynylthiophene-2-carboxylic acid **6a** (bearing a terminal triple bond) and of 3-(phenylethynyl)thiophene-2-carboxylic acid **6b** (bearing a triple bond substituted with a phenyl group) (Scheme 6). Interestingly, with these substrates, only the corresponding 6-membered heterocycles were obtained, with no formation of the 5-membered regioisomers. On the other hand, as expected, 7*H*-thieno[2,3-*c*]thiopyran-7-thiones **7a,b** (dithionated products) or 7*H*-thieno[2,3-*c*]thiopyran-7-ones **8a,b** (monothionated products) were selectively formed using either 1 equiv of the LR for 1 h or 0.5 equiv of the LR for 20 min., as shown in Scheme 6.



Scheme 6. Synthesis of 7*H*-thieno[2,3-*c*]thiopyran-7-thiones **7a,b** and 7*H*-thieno[2,3-c]thiopyran-7-ones **8a,b** by dithionation or monothionation – cycloisomerization of 3-alkynylthiophene-2-carboxylic acids **6a,b**.

***In-vitro* evaluation of the herbicidal activity of the sulfurated heterocycle derivatives**

In the continuous search of new chemicals with phytotoxic activity, for the development of new classes of synthetic herbicides, some of synthesized sulfurated heterocycles have been assayed at a relatively broad concentration range (0–400 µM). The phytotoxic potential of these compounds was evaluated on seedlings growth and development of the model species *A. thaliana,* largely used in phytotoxic bioassays because of its high sensitivity to both natural and synthetic toxins.[8] This first step is essential to identify the key concentrations (i.e. ED_{50}, LD_{50} etc.) of each molecule to be later used to identify their potential target and mode of action.

Interestingly, all the molecules assayed significantly affected, at different extent, all the morpho-physiological parameters monitored. In particular, with respect to the reduction of leaf number (LN), a high inhibitory effect was exerted by **3e** and **7a**, which showed an ED_{50} of ca. 73.6 µM (Figure 1 and Table 1). Compounds **2d, 4b**, and **4c** were able to reduce LN at concentrations > 200 µM, while **2d** did not affect it (Figure 1 and Table 1). On the other hand, all the compounds tested strongly affected the Fresh Weight (FW), with ED_{50} values ranging from 4.81 µM (for **2d**) to 63.7 µM (for **4b**) (Figure 1 and Table 1). The reduction in LN accompanied by a strong decrease in FW suggests that the compounds are able to reduce both plant

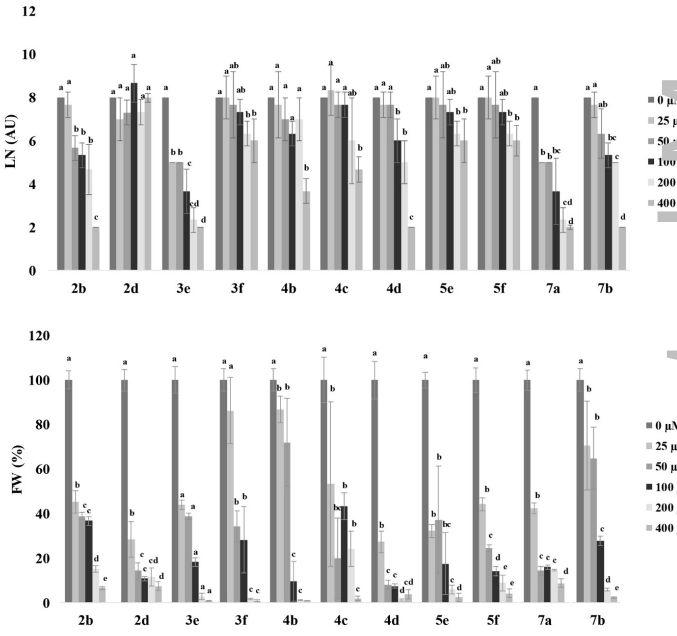


Figure 1. Leaf number (LN) and fresh weight (FW) of *A. thaliana* seedlings treated for 14 days with increasing doses (0-400 µM) of different sulfurated heterocycle derivatives. Data were expressed as percentage compared to control and analyzed through one-way ANOVA using the LSD as post-hoc ($P \leq 0.05$). Bars indicate SD. N=4.

Table 1. ED_{50} (μM) values of leaf number (LN) and fresh weight (FW) of *A. thaliana* estimated by the log-logistic equations in response to different doses of the different sulfurated heterocycle derivatives.[a]

Compound	LN	FW
	ED_{50} (μM)	
2b	191.44 (14.01)^{c}	13.78 (1.7)^{d}
2d	> 400^{a}	4.81 (2.4)^{e}
3e	73.55 (10.8)^{d}	24.65 (3.1)^{c}
3f	> 400^{a}	45.45 (8.3)^{b}
4b	> 400^{a}	63.65 (4.5)^{a}
4c	> 400^{a}	22.88 (5.6)^{c}
4d	236.93 (13.21)^{b}	12.35 (2.15)^{d}
5e	> 400^{a}	12.43 (1.04)^{d}
5f	> 400^{a}	16.19 (1.5)^{c}
7a	73.55 (11.82)^{d}	10.07 (3.15)^{de}
7b	212.4 (11.6)^{c}	48.81 (8.4)^{ab}

[a] Different letters along the column indicate statistically significant differences among the treatments. Data were analyzed through one-way ANOVA using the LSD test as post-hoc ($P \leq 0.05$). Values between brackets indicate SD. N=4.

growth and development. Similar results were observed in Arabidopsis seedlings treated with three synthetic coumarin derivatives, which differentially affected shoot development, suggesting that, despite their structural similarity, they could have a different mode of action on plants.[9]

In addition, all compounds significantly affected, to a different extent, the photosynthetic pigment content (chla, chlb and carotenoids). These effects, already evident for almost all molecules at the lowest concentration (25 μM), were extremely marked at concentrations higher than 50 μM for all the sulfurated heterocycle derivatives (Figure 2). Moreover, a similar response was observed for the lipid peroxidation that was significantly stimulated by all molecules at concentrations higher than 50 μM, reaching, at the highest concentration (400 μM), an increment ≃ 2.5 folds compared to the control (Figure 3). The increase in lipid peroxidation accompanied by the decrease in pigment content suggested that plants were subjected to oxidative stress. Heavy metals, excessive herbicide use and allelochemicals may trigger oxidative stress in plants, inducing the intracellular over–production of reactive oxygen species (ROS).[10] The increase in ROS production causes drastic changes in membrane permeability, degradation of both unsaturated membrane lipids and photosynthetic pigments, protein degradation and, consequently, plant growth and development inhibition,[11] as observed in our experiments. Recently, similar effects were observed in Arabidopsis plants, treated with *trans*-caryophyllene, which caused a strong reduction in pigment content and PSII (Photosystem II) efficiency, mainly due to physical damages to the antenna complexes.[12] This PSII inefficiency was probably due to the strong reduction in carotenoid content and the inability of plants to activate the xanthophylls cycle. In fact, as reported by Ramel and coworkers,[13] carotenoids, which are strongly involved in plant defence against toxicity induced by ROS accumulation, play a pivotal role in protection against photoinhibition and photodegradation phenomena.[14]

The alteration in shoot growth and development could be due to a direct effect, exerted by the molecules on the above-ground organs, as well as an indirect effect on the root system, compromising its ability in water and nutrient uptake. Therefore, the effects of these molecules on root morphology and, in particular, on primary root length (PRL) and lateral root number, were evaluated. Primary root length (PRL) was significantly affected by all compounds, showing ED_{50} values ranging from 63.5 μM (for **5e**) to 266.2 μM (for **3f**) (Figure 4 and Table 2), except for **3e**, which presented ED_{50} values higher than 400 μM (Figure 4 and Table 2). Except for **3e**, **3f**, and **7b**, which caused a 50% reduction of the number of lateral roots (NRL) at concentrations higher than 50 μM [ranging from 60.4 μM (for **7b**) to 185 μM (for **3e**)], all other molecules strongly affected this parameter at the lowest concentrations (ED_{50} values < 50 μM) (Figure 4 and Table 2). All the sulfurated heterocycles tested caused a strong reduction in root growth, accompanied by an absence in lateral roots and root hairs (Figures 4 and 5, and Table 2).

The ability of synthetic molecules and allelochemicals to alter the primary root growth and the number and length of laterals has been widely documented.[9,15] Recent studies reported that natural phytotoxic farnesene inhibited primary root length and lateral root formation through the alteration of cell mitosis and root meristem development.[12,16] Moreover, root morphological changes could be due to the ability of some phytotoxic compounds to alter the hormones balance such as IAA (indole-3-acetic acid), involved in root growth regulation.[17] Probably, an alteration on IAA could explain the anatomical modification induced by 100 μM of the sulfurated heterocyclic derivatives on the root tip of the primary roots (Figure 5). Higher concentrations completely deformed the root giving no hint on the potential target of the molecules. Almost all molecules, except for **2b, 2d** and **3f**, also caused bold roots due to the absence of root hairs. Similar effects were observed on Arabidopsis roots treated with citral[18] and farnesene,[16] which induced an alteration of the ethylene/auxin hormonal balance.

In addition, molecules **4b**-**5f** induced a cork-screw root shape. This phenomenon, called "handedness", may interest both shoots and roots,[16,19] and in relation to the direction is defined “fixed” "left or right handedness" or random.[20] Compounds **4c** and **5e** induced a clear right handedness, whereas **4b**, **4d** and **5f** caused a random root torsion (Figure 5). Mutants with fixed handedness exhibited defects in microtubules arrangement, whereas those with random direction seemed to interfere with auxin transport systems.[12,21] Compounds **3f** and **7b** caused a radial generalized swelling in the root meristem and in the elongation zone, respectively, characterized by an abnormal shape (Figure 5).

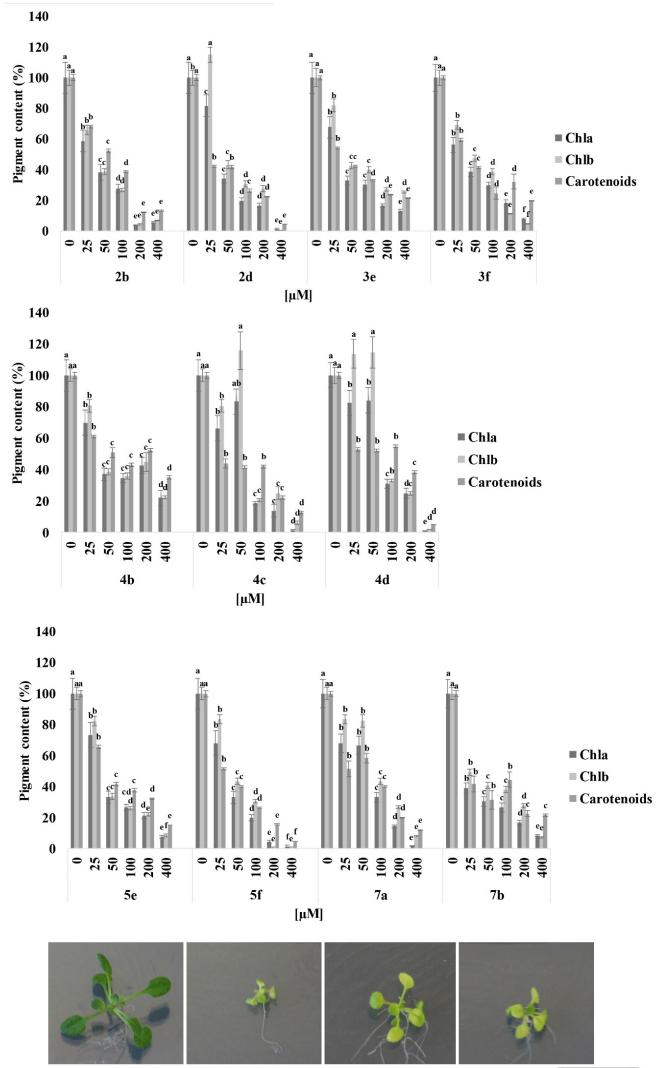


Figure 2. Pigment content of *A. thaliana* seedlings treated for 14 days with increasing doses (0-400 µM) of different sulfurated heterocycle derivatives. Data were analyzed through one-way ANOVA using the LSD test as post-hoc ($P \leq 0.05$). Bars indicate SD. N=4. In the photos below the graphs, some examples of the reduction in plant pigmentation (treatment 100 µM). (From the left to the right: Control, **5e**, **4b**, **5f**).

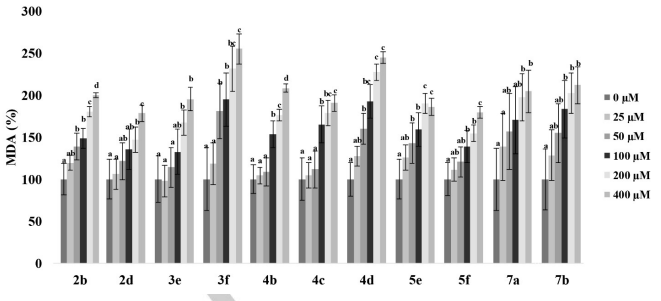


Figure 3. Lipid peroxidation (MDA) of *A. thaliana* seedlings treated for 14 days with increasing doses (0-400 µM) of different sulfurated heterocycle derivatives. Data were analyzed through one-way ANOVA using the LSD test as post-hoc ($P \leq 0.05$). Bars indicate SD. N=4.

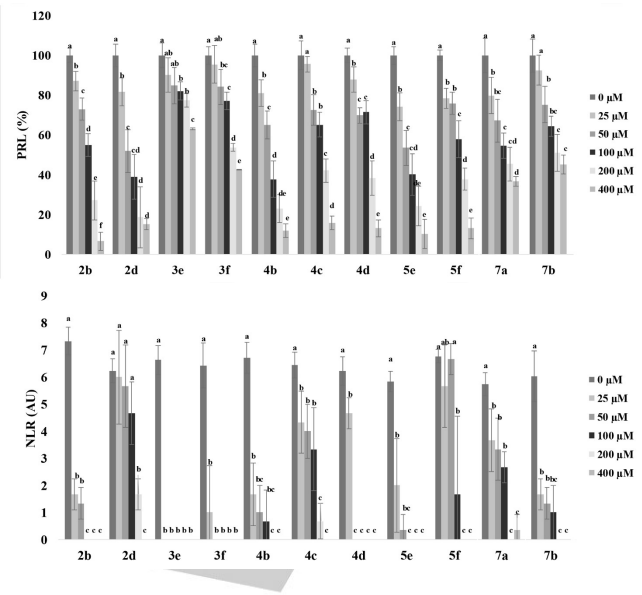


Figure 4. Primary root length (PRL) and number of lateral roots (NLR) of *A. thaliana* seedlings treated for 14 days with increasing doses (0-400 µM) of different sulfurated heterocyclic derivatives. Data were expressed as percentage compared to the control and analyzed through one-way ANOVA using the LSD's test as post-hoc ($P \leq 0.05$). Bars indicate SD. N=4.

Table 2. ED_{50} (µM) values of primary root length (PRL) and number of lateral root (NLR) of *A. thaliana* estimated by the log-logistic equations in response to increasing doses of the different sulfurated heterocycle derivatives.[a]

Compound	PLR	NLR
	ED_{50} (µM)	
2b	106.24 (± 9.4)^{f}	12.63 (1.9)^{e}
2d	63.59 (± 9.96)^{h}	9.02 (0.5)^{f}
3e	> 400^{a}	185 (3.2)^{a}
3f	266.24 (± 11.39)^{b}	68.44 (11.7)^{b}
4b	79.42 (± 4.73)^{g}	10.07 (1.5)^{ef}
4c	142.46 (± 12.93)^{d}	44.25 (3.4)^{c}
4d	150.15 (± 13.16)^{d}	45.93 (2.9)^{c}
5e	63.48 (± 5.07)^{h}	0.0005 (0.0004)^{g}
5f	126.98 (± 11.4)^{e}	24.27 (0.57)^{d}
7a	151.83 (± 17.01)^{d}	47.1 (3.2)^{c}
7b	236.48 (± 11.83)^{c}	60.41 (0.4)^{b}

[a] Different letters along the column indicate statistically significant differences among the treatments. Data were analyzed through one-way ANOVA using the LSD test as post-hoc ($P \leq 0.05$). Values between brackets indicate SD. N=4.

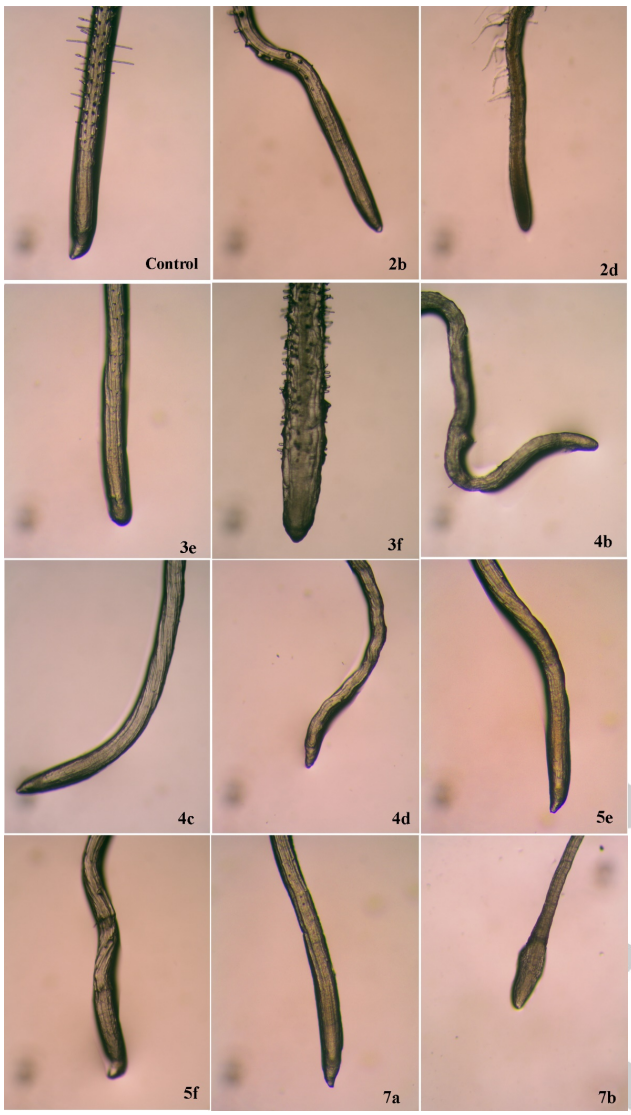


Figure 5. Root tip anatomy of *Arabidopsis thaliana* treated with 100 µM of different sulfurated heterocycle derivatives for 14 days. Microscope magnification 20X.

This phenomenon, accompanied by root inhibition, was already observed in corn seedlings treated with colchicine and oryzalin, two microtubules stabilizer.[22] Moreover, recent studies demonstrated that the swelling phenomenon is mainly due to the enlargement of the stele tissue and cortex cells as well as to an increased thickness of cell wall due to an increment of cellulose deposition.[23]

Finally, **2d** did not induce evident anatomical alterations, but root tip showed wilt hairs and a brownish color. This effect was already observed in roots which are experiencing cell death mediated by a ROS burst.[24] For this reason, the cell viability of seedlings treated with the sulfureted heterocycles, at their ED_{50} values, were assayed through the trypan blue dye exclusion test. The test is based on the fact that living cells are able to exclude this dye, while dead cells are not.[25] The results highlighted that, among all the molecules tested, only **2d** and **5e** were able to induce cell death in root meristem (Figure 6).

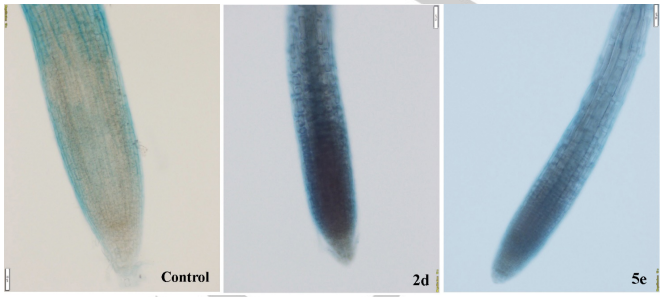


Figure 6. Cell death on *A. thaliana* root meristem treated with the ED_{50} dose of two sulfurated heterocycle derivatives, **2d** and **5e**. N = 4. The bar reported on the side of each photo correspond to 50 µm.

Conclusions

In conclusion, we have developed a simple and convenient approach to sulfurated heterocyclic derivatives by a tandem process consisting of thionation of 2-alkynylbenzoic acids followed by cycloisomerization, using the Lawesson's reagent (LR) under microwave irradiation. Depending on the amount of the LR and on the reaction time, it has been possible to selectively convert the substrates to either disulfurated heterocycles (benzo[*c*]thiophene-1(3*H*)-thiones **2** or 1*H*-isothiochromene-1-thiones **3**) or monosulfurated heterocycles (benzo[*c*]thiophen-1(3*H*)-ones **4** or 1*H*-isothiochromen-1-ones **5**). The regiochemical output of the process strongly depended on the substitution pattern of the substrates; in particular, products **2** and **4** (from a 5-*exo-dig* cyclization mode) were obtained from substrates bearing an aryl group on the triple bond (either unsubstituted or substituted with an electron-withdrawing group). On the other hand, **3** and **5** (from 6-*endo-dig* cyclization) were formed from substrates bearing, on the triple bond, an alkyl group or an aryl group substituted with a π-donating group. In the case of 2-alkynylthiophene-2-carboxylic acids **6**, the formation of the 6-membered heterocycles **7** and **8** only was observed.

Given the importance of sulfurated heterocycles in agrochemistry, these compounds have also been tested as potential herbicides. The results obtained on seedlings growth and development of the model species *A. thaliana,* confirmed that these molecules are very interesting and promising. Indeed, almost all molecules were able to affect both shoot and root growth and development, as a consequence of a systemic or an indirect effect, altering their morphology. Moreover, the results evidenced that structurally different compounds may induce diverse plant response, thus suggesting different modes of action. Further studies will be focused on the evaluation of the herbicidal potential on both crops and weeds using more complex systems, such as microcosms, and different ways of application such as spray and/or irrigation. Finally, these molecules will be assayed on living cells (human and/or rat

and/or mouse etc.) to evaluate their potential dangerousness towards human and animal health.

Experimental Section

General

Solvents and reagents were commercially available (Sigma-Aldrich). Microwave-assisted syntheses were performed using a microwave oven CEM Discover in sealed reaction vessels. The temperature was monitored using a vertically focused IR temperature sensor. In order to have a homogenous system, all the batches were started with a ramp time of 120 seconds, and when the temperature program was completed, a cooling period of 10 minutes was included. Mass spectra were obtained on an Agilent 6540 UHD accurate-mass Q-TOF spectrometer equipped with a Dual AJS ESI source working in positive mode. NMR spectra (^{1}H NMR at 500 MHz, ^{13}C NMR at 126 MHz) were recorded with Varian instruments; chemical shifts are reported in ppm relative to $CDCl_3$ (7.26 ppm). Merck silica gel 60-F254 precoated aluminum plates were used for thin-layer chromatographic separations. Flash chromatography was performed on Merck silica gel (200–400 mesh). Preparative separations were carried out by a MPLC Büchi C-601 by using Merck silica gel 0.040–0.063 mm. Evaporation refers to removal of the solvent under reduced pressure.

Preparation of substrates

Substrates **1a-f** were prepared as we already reported.[5] Substrates **6** were prepared from commercially available 3-bromothiophene-2-carboxylic acid by esterification followed by Sonogashira coupling and hydrolysis, as reported below.

Esterification of 3-bromothiophene-2-carboxylic acid

To a solution of 3-bromothiophene-2-carboxylic acid, (5.0 g, 24.1 mmol), dissolved in MeOH (121 mL), was added trimethylsilyl chloride (6.13 mL, 88.3 mmol) under nitrogen. The resulting mixture was heated under reflux for 12 h. After evaporation of the solvent, the residue was purified by flash chromatography (8:2 cyclohexane-ethyl acetate) to give methyl 3-bromothiophene-2-carboxylate as a white solid (yield: 5.05 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.46 (d, J = 5.3 Hz, 1 H), 7.09 (d, J = 5.3 Hz, 1 H), 3.90 (s, 3 H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 160.5, 131.5, 132.2, 124.2, 117.2, 51.5 ppm. Physical and spectroscopic data were in agreement with those reported in the literature.[26]

Sonogashira coupling between methyl 3-bromothiophene-2-carboxylate and terminal alkynes

A sealed tube (10 mL capacity) was charged with a solution of PPh_3 (60 mg, 0.23 mmol), $PdCl_2(PPh_3)_2$ (161 mg, 0.23 mmol) and NEt_3 (6 mL). The resulting mixture was heated at 60 °C for 2 h. After cooling, methyl 3-bromothiophene-2-carboxylate (1.0 g, 4.55 mmol), the alkyne (trimethylsilylacetylene: 0.69 mL, 5.0 mmol; phenylacetylene: 0.55 mL, 5.0 mmol) and CuI (29 mg, 0.15 mmol), were sequentially added, and the resulting mixture was heated at 60 °C for 2 h. After evaporation of the solvent, the residue was purified by flash chromatography (95:5 cyclohexane/ethyl acetate) to give the coupling product.

Methyl 3-((trimethylsilyl)ethynyl)thiophene-2-carboxylate. Yield: 0.945 g, 88%, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.41 (d, J = 5.1 Hz, 1H), 7.14 (d, J = 5.1 Hz, 1H), 3.90 (s, 3H), 0.28 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 162.6, 134.9, 129.0, 128.7, 125.9, 105.6, 96.0, 51.7. Physical and spectroscopic data were in agreement with those reported in the literature.[27]

Methyl 3-(phenylethynyl)thiophene-2-carboxylate. Yield: 0.986 g, 90%, yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.62–7.57 (m, 2 H), 7.45 (d, J = 5.1 Hz, 1 H), 7.36–7.34 (m, 3 H), 7.20 (d, J = 5.1 Hz, 1 H), 3.93 (s, 3 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 160.5, 133.8, 133.2, 132.3, 128.4, 128.3, 127.4, 104.8, 86.9, 80.6, 51.4 ppm. Physical and spectroscopic data were in agreement with those reported in the literature.[28]

Hydrolysis of methyl 3-((trimethylsilyl)ethynyl)thiophene-2-carboxylate and methyl 3-(phenylethynyl)thiophene-2-carboxylate leading to 6a and 6b, respectively

To a solution of methyl 3-((trimethylsilyl)ethynyl)thiophene-2-carboxylate (500 mg, 2.09 mmol) or methyl 3-(phenylethynyl)thiophene-2-carboxylate (500 mg, 2.06 mmol) dissolved in a 2:1 (v/v) mixture of MeOH/EtOH (30 mL), was added 2N NaOH (7 mL), and the resulting mixture solution was stirred at rt for 2 h. When TLC analysis showed that all the starting material was converted, the solution was concentrated under reduced pressure. The residue was dissolved in water (30 mL) and then washed with diethyl ether (2 x 20 mL). After acidification to pH = 4-5, the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and, after filtration, concentrated under reduced pressure to give crude 3-ethynylthiophene-2-carboxylic acid **6a** or 3-(phenylethynyl)thiophene-2-carboxylic acid **6b**, which were used as such for the subsequent thionation-cycloisomerization reactions.

*3-Ethynylthiophene-2-carboxylic acid **6a**.* Yield: 0.255 g, 80%, orange powder, m.p. 115-119 °C. ^{1}H NMR (500 MHz, cdcl$_3$) δ 7.55 (d, J = 5.1 Hz, 1H), 7.22 (d, J = 5.1 Hz, 1H), 3.52 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 163.7, 135.9, 128.5, 128.1, 126.2, 87.6, 74.9. Physical and spectroscopic data were in agreement with those reported in the literature.[27]

*3-(Phenylethynyl)thiophene-2-carboxylic acid **6b**.* Yeld: 0.414 g, 88%, grey powder, m.p. 146-148 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.61 – 7.53 (m, 3H), 7.38 – 7.30 (m, 3H), 7.25 (d, J = 5.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 166.86, 132.84, 132.55, 132.14, 132.00, 129.01, 128.83, 128.55, 122.88, 96.57, 83.88 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{13}H_9O_2S)^+$: 229.0318; found, 229.0326.

General procedure for the tandem thionation-heterocyclization of substrates 1a-f and 6a,b to give sulfureted heterocycles

A sealed tube (10 mL capacity) was charged with a solution of **1** (0.45 mmol) and the Lawesson's reagent (0.45 mmol for the synthesis of dithionated products **2a-d**, **3e**, **3f**, **7a**, and **7b**; 0.22 mmol for the synthesis of monothionated products **4a-d**, **5e**, **5f**, **8a**, and **8b**) in CH_2Cl_2 (3 mL). The mixture was irradiated under microwave conditions at 300 W and 100 °C for the required time (1 h for the synthesis of **2a-d**, **3e**, and **3f**; 20 min for **4a-d** and **8a-b**; 30 min for **5e** and **5f**. After cooling, the reaction mixture was concentrated under reduced pressure, and products were purified by MPLC (medium pressure liquid chromatography) using 9:1 cyclohexane/CH_2Cl_2 as eluent.

Characterization data for dithionated heterocycles **2a-d**, **3e**, **3f** was reported in our preliminary communication.[5] All other products were fully characterized as reported below.

*(Z)-3-Benzylidenebenzo[c]thiophen-1(3H)-one (**4a**).* Yield: 90 mg, 84%. Yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, J = 8.0 Hz, 1 H), 7.86

(d, *J* = 7.9 Hz, 1 H), 7.68 (t, *J* = 7.6 Hz, 1 H), 7.64–7.59 (m, 3 H), 7.49 (t, *J* = 7.5 Hz, 1 H), 7.45 (t, *J* = 7.7 Hz, 2 H), 7.35 (t, *J* = 7.4 Hz, 1 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 193.51, 144.50, 135.43, 133.58, 133.08, 132.85, 131.19, 129.80, 129.09, 129.03, 128.82, 128.64, 124.95, 123.77, 120.91 ppm; HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{15}H_{11}OS)^+$: 239.0525; found, 239.0513.

(Z)-3-Benzylidene-4-methylbenzo[c]thiophen-1(3H)-one (***4b***) Yield: 91 mg, 84%. Orange oil. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 7.79 (d, *J* = 7.6 Hz, 1 H), 7.74 (s, 1 H), 7.57 (d, *J* = 7.5 Hz, 2 H), 7.50 (d, *J* = 7.3 Hz, 1 H), 7.44 (t, *J* = 7.7 Hz, 2 H), 7.40–7.32 (m, 2 H), 2.82 (s, 3 H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 193.97, 141.20, 137.31, 136.64, 135.19, 135.01, 134.25, 130.14, 129.73, 128.90, 128.68, 128.46, 23.65 ppm; HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{16}H_{13}OS)^+$: 253.0681; found, 253.0678.

(Z)-3-Benzylidene-6-chlorobenzo[c]thiophen-1(3H)-one (***4c***). Yield: 87 mg, 82%, Amorphous yellow powder, m.p. 95-97 °C. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 7.91 (d, *J* = 8.4 Hz, 1 H), 7.81 (d, *J* = 1.8 Hz, 1 H), 7.62 (dd, *J* = 8.4, 1.8 Hz, 1 H), 7.58 (d, *J* = 7.4 Hz, 2 H), 7.57 (s, 1 H), 7.45 (t, *J* = 7.7 Hz, 2 H), 7.36 (t, *J* = 7.4 Hz, 1 H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 192.05, 142.63, 135.59, 135.13, 134.38, 133.59, 130.13, 129.81, 129.14, 125.74, 123.48, 121.90 ppm; HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{15}H_{10}ClOS)^+$: 273.0135; found, 273.0138.

(Z)-3-(4-Fluorobenzylidene)benzo[c]thiophen-1(3H)-one (***4d***). Yield: 90 mg, 84%, Amorphous grey powder, m.p. 84-86 °C. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 7.97 (d, *J* = 8.5 Hz, 1 H), 7.86 (d, *J* = 7.6 Hz, 1 H), 7.68 (t, *J* = 7.6 Hz, 1 H), 7.58 (dd, *J* = 8.8, 5.3 Hz, 2 H), 7.56 (s, 1 H), 7.50 (t, *J* = 7.5 Hz, 1 H), 7.14 (t, *J* = 8.5 Hz, 2 H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 206.95, 193.07, 163.62, 144.26, 133.51, 131.44 (d, *J* = 8.2 Hz), 129.03, 123.64 (d, *J* = 25.4 Hz), 120.70, 116.15 (d, *J* = 21.8 Hz) ppm; HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{15}H_{10}FOS)^+$: 257.0431; found, 257.0423.

3-(4-Methoxyphenyl)-1H-isothiochromen-1-one (***5e***). Yield: 85 mg, 80%, Amorphous orange powder, m.p. 117-119 °C. ^{1}H NMR (500 MHz, cdcl$_3$) *δ* = 8.29 (dd, *J* = 8.0, 0.5 Hz, 1H), 7.85 – 7.80 (m, 2H), 7.69 (td, *J* = 7.5, 1.3 Hz, 1H), 7.49 – 7.43 (m, 2H), 6.99 – 6.95 (m, 2H), 6.83 (s, 1H), 3.87 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 162.63, 161.22, 153.87, 138.06, 134.95, 129.78, 127.81, 126.97, 125.83, 124.69, 120.30, 114.39, 100.38, 55.56 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{16}H_{13}O_2S)^+$: 269.0631; found, 269.0632.

3-Propyl-1H-isothiochromen-1-one (***5f***). Yield: 85 mg, 78%, brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) *δ* = 7.94 (d, *J* = 8.2 Hz, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 1H), 6.72 (s, 1H), 2.60 (t, *J* = 7.5 Hz, 3H), 1.82 – 1.71 (m, 3H), 1.00 (t, *J* = 7.4 Hz, 4H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 163.21, 157.61, 135.19, 132.85, 132.75, 132.28, 129.86, 128.72, 125.68, 106.24, 35.43, 20.61, 13.57 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{12}H_{13}OS)^+$: 205.0681; found, 205.0680.

7H-Thieno[2,3-c]thiopyran-7-thione (***7a***). Yield: 108 mg, 89%, Amorphous red powder, m.p. 88-90 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ* = 7.95 (d, *J* = 5.3 Hz, 1H), 7.65 (d, *J* = 9.4 Hz, 1H), 7.52 (d, *J* = 9.4 Hz, 1H), 7.38 (d, *J* = 5.3 Hz, 1H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 196.79, 148.88), 139.52), 138.36, 134.43, 128.38, 121.19 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_7H_5S_3)^+$: 184.9547; found, 184.9547.

5-Phenyl-7H-thieno[2,3-c]thiopyran-7-thione (***7b***). Yield: 103 mg, 90%, Amorphous red powder, m.p. 100-102 °C. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 7.95 (d, *J* = 5.3 Hz, 1H), 7.76 (s, 1H), 7.63 – 7.57 (m, 2H), 7.50 – 7.45 (m, 3H), 7.41 (d, *J* = 5.3 Hz, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 196.94, 151.04, 147.21, 141.31, 138.63, 135.55, 130.24, 129.52, 128.62, 127.10 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{13}H_9S_3)^+$: 260.9860; found, 260.9868.

7H-Thieno[2,3-c]thiopyran-7-one (***8a***). Yield: 77 mg, 70%, orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) *δ* = 7.84 (d, *J* = 5.2 Hz, 1H), 7.67 (d, *J* = 9.4 Hz, 1H), 7.53 (d, *J* = 9.4 Hz, 1H), 7.39 (d, *J* = 5.2 Hz, 1H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): *δ* = 179.40, 146.92, 134.37, 134.21, 129.27, 127.92, 117.89, 114.28 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_7H_5OS_2)^+$: 168.9776; found, 168.9782.

5-Phenyl-7H-thieno[2,3-c]thiopyran-7-one (***8b***). Yield: 96 mg, 90%, red-dark oil. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* = 7.85 (d, *J* = 5.1 Hz, 1H), 7.64 – 7.59 (m, 2H), 7.54 (s, 1H), 7.49 – 7.44 (m, 3H), 7.36 (d, *J* = 5.1 Hz, 1H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 179.75, 147.69, 145.57, 136.81, 134.72, 131.98, 129.80, 129.32, 128.24, 127.20, 115.61 ppm. HRMS-ESI [(M+H)$^+$]: *m/z* calcd for $(C_{13}H_9OS_2)^+$: 245.0089; found, 245.0093.

In-vitro evaluation of the herbicidal activity of sulfurated heterocycles

Effects on root morphology

Seed sterilization, vernalization and germination were carried out as previously reported.[29] Briefly, seeds of *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia (Col-0) were sterilized for 3 min in 50% EtOH and 0.5% NaOCl with Triton X-100 at 0.01% and then washed three times in distilled water. After sterilization, seeds were maintained in 0.1% agar at 4 °C for 72 h to allow vernalization. Then, 24 seeds were sown in square Petri dishes (100 x 150 mm) containing plant agar (0.8% w/v) medium enriched with a mixture of micro and macronutrients (Murashige-Skoog, Sigma-Aldrich) supplemented with 1% sucrose. The plates were placed vertically in the growth chamber to encourage geotropic growth of roots. Growing conditions were 22 ± 2 ° C and light intensity of 75 mol $m^{-2}s^{-1}$. Immediately after germination, five uniform, 4-d old seedlings were transferred to a single plate and grown for 14 d with the same medium containing 0, 25, 50, 100, 200, 400 µM of each heterocyclic derivative. The molecules were previously dissolved in EtOH and the same amount of EtOH (0.1% v/v) was added to the control (0 µM). At the end of the experiments, the whole root system was imaged by scanning (STD 1600, Régent Instruments Inc., Quebec, Canada) and primary root length (PRL) and number of lateral roots (NLR) were measured using WinRhizo Pro system v. 2002a (Instruments Régent Inc., Quebec, Canada).

Effects on shoot development and pigments content

To evaluate the phytotoxic potential of the selected molecules on plant growth and development, different parameters were considered. In particular, total fresh weight (FW), leaf number (LN) and pigments content were evaluated. Regarding the last parameter, total amounts of chlorophyll a, chlorophyll b, and carotenoids were analyzed and calculated according to Wellburn.[30] The pigments content was evaluated according to the following equations:

$$Chl_a\ (\mu g) = (15.65\ (DO_{666} - DO_{750}) - 7.34\ (DO_{653} - DO_{750}))*V$$

$$Chl_b\ (\mu g) = (27.05\ (DO_{653} - DO_{750}) - 11.21\ (DO_{666} - DO_{750}))*V$$

$$Ct\ (X+C)\ (\mu g) = (1000\ (DO_{470} - DO_{750}) - 2.86\ Chl_a - 129.2\ Chl_b)/221)*V$$

Where, DO_{470}, DO_{666}, DO_{653}, DO_{750} represent the optical density of the sample readed at 470, 666, 653 and 750 nm, respectively. Pigments content was calculated as µg / g of DW and then expressed as percentage compared to control.

Lipid peroxidation

Lipid peroxidation was determined on ten old seedlings of *A. thaliana* by measurement of malonyldialdehyde (MDA) content as previously described by Hodges et al.[31] with the corrections proposed by Landi.[32] After treatment, plant material (100 mg) was homogenized in 80% ethanol and centrifuged at 3000×g for 10 min at 4 °C. The supernatant was collected and incubated at 95 °C with 20% TCA containing 0.01% dibutylhydroxytoluene (BHT), in presence or absence of 0.5% thiobarbituric acid (TBA). The equivalents of MDA, calculated as nmol/mL and then expressed as percentage compared to control, were calculated using the following equations:

$A = [(Abs_{532+TBA}) – (Abs_{600+TBA}) - (Abs_{532-TBA} – Abs_{600-TBA})]$

$B = [(Abs_{440\ +\ TBA} - Abs_{600+TBA}) * 0.0571]$

MDA equivalents (nmol / ml) = $(A-B)/157000 * 10^6$

Trypan blue staining

Arabidopsis root cell death was evaluated as previously described by Araniti et al.[24a] For the experiments, the ED_{50} values previously calculated for PRL parameter were used. Roots of 20 untreated and treated seedlings (for each replicate), were soaked in an aqueous solution of Trypan blue (0.5 % w/v) and incubated in dark conditions for 5 min. After incubation, roots were rinsed several time in phosphate-buffered saline (PBS) (pH 7.4) to eliminate the dye excess. Immediately after the washing, root apexes were visualized under an epifluorescence microscopy (Olympus BX53). Root cells characterized by deep blue stain indicate the presence of root cell death.

Statistical analysis

To evaluate the phytotoxic effects of the different molecules, a completely randomized design with four replications was adopted. Data were evaluated for normality (Kolmogorov-Smirnov test) and tested for homogeneity of variances (Levene's test). The statistical significance of differences among group means were estimated by analysis of variance (one way-ANOVA) followed by LSD test. All statistical analyses were conducted using SPSS *ver*. 6.1 software (Insightful Corporation, USA). The responses of FW, LN, PRL, NLR to different doses of the sulfurated heterocycle derivatives were evaluated by a nonlinear regression model using the following equation, largely employed for the evaluation of the phytotoxic potential of both natural and synthetic molecules:[33]

$y = C+\{D-C/1+e\text{^}[B \ln(x/ED_{50})]\}$

Where C denotes the expected response at indefinitely large concentrations, D denotes the control mean response, ED_{50}, denotes a specific parameter which defines the dose required to reduce 50% of the total response, B denotes the rate of change around the ED_{50}. The expected response is assumed to be zero for infinitely large concentrations (we assume C=0).

The ED_{50} comparison among the different molecules was performed by one-way ANOVA followed by LSD test ($P \leq 0.05$) using the ED_{50} as a variable with the molecule as main factor.

Acknowledgements

Thanks are due to Prof. Antonio Palumbo Piccionello (Department of Biological, Chemical and Pharmaceutical Science and Technology – STEBICEF, University of Palermo, Italy) for HRMS measurements.

Conflict of interest

The authors declare no conflict of interest.

Keywords: cycloisomerization • herbicides • heterocycles • Lawesson's reagent • microwave-assisted synthesis • thionation

[1] P. Hazell, S. Wood, *Philos. Trans. R. Soc. B-Biol. Sci.* **2008**, *363*, 495-515.
[2] F. P. Carvalho, *Food Energy Secur.* **2017**, *6*, 48–60.
[3] a) M. W. Aktar, D. Sengupta D, A. Chowdhury *Interdiscip. Toxicol.* **2009**, *2*, 1-12; b) J. Popp, K. Peto, J. Nagy, *Agron. Sustain. Dev.* **2013**, *33*, 243–255.
[4] D. Ponnam, G.-F. Yang, *Top. Curr. Chem.* **2017**, *375*, Article No. 82.
[5] S. V. Giofrè, R. Romeo, R. Mancuso, N. Cicero, N. Corriero, U. Chiacchio, G. Romeo, B. Gabriele, *RCS Adv.* **2016**, *6*, 20777-20780.
[6] Classical methods for the preparation of benzo[*c*]thiophene-1(3*H*)thiones include the dithionation of isobenzofuran-1(3*H*)-one derivatives with P_2S_5 or P_4S_{10} and the reaction of ω-keto amides with the Lawesson's reagent under conventional heating. See: a) R. Meyer J. Szanecki, *Chem. Ber.* **1900**, *33*, 2577-2584; b) T. J. Dickerson, N. N. Reed, J. J. LaClair, K. D. Janda, *J. Am. Chem. Soc.* **2004**, *126*, 16582-16586; c) T. Nishio, *Helv. Chim. Acta* **1998**, *81*, 1207-1214.
[7] 1*H*-Isothiochromene-1-thione derivatives have been previously obtained by thionation of 1*H*-isothiochromen-1-one with P_4S_{10} or the Lawesson's reagents under conventional heating (see, for example: J. Voss, G. Kupczik, H. Stahncke, *J. Chem. Res.* **2009**, 283-285), the reaction of 3-iodomethyl-2-benzothiophene-1(3*H*)-thiones with sodium hydrogencarbonate in refluxing MeCN (S. Fukamachi, H. Konishi, K. Kobayashi, *Heterocycles* **2009**, *78*, 169-176), and the treatment of 2-chloro-*N*-cyanomethyl-*N*-ethyl-5-nitrobenzamide with NaH and CS_2 (W. Dölling, M. Biedermann, H Hartung, *Eur. J. Org. Chem.* **1998**, 1237-1242).
[8] M. Pennacchio, L. V. Jefferson, K. Havens, *J. Chem. Ecol.* **2005**, *31*, 1877-1885.
[9] F. Araniti, R. Mancuso, A. Lupini, S. V. Giofrè, F. Sunseri, B. Gabriele, M. R.Abenavoli, Molecules **2015**, *20*, 17883-17902.
[10] a) X. S. Song, C. L. Tiao, K. Shi, W. H. Mao, J. O. Ogweno, Y. H. Zhou, J. Q. Yu, *Plant Growth Regul.* **2006**, *49*, 85-93; b) R. Cruz-Ortega, A. Lara-Núñez, A. L. Anaya, *Plant Signal. Behav.* **2007**, *2*, 269-270.
[11] a) M. N. Merzlyak, G. A. F. Hendry, *Proc. R. Soc. Edinb. Sect. B-Biol. Sci.* **1994**, *102*, 459-471.; b) S. Verma, R. S. Dubey, *Plant Sci.* **2003**, *164*, 645-655.; c) K. Taïbi, F. Taïbi, L. A. Abderrahim, A. Ennajah, M. Belkhodja, J. M. Mulet, South Afr. J. Botany **2016,** *105*, 306-312.
[12] F. Araniti, L. Bruno, F. Sunseri, M. Pacenza, I. Forgione, M. B. Bitonti, M. R. Abenavoli, *Plant Physiol. Biochem.* **2017**, *121*, 14-20.
[13] F. Ramel, S. Birtic Cuinè, C. Triantaphylides, J.L. Ravanat, M. Havaux, *Plant Physiol.* **2012**, *158*, 1267–1278.

[14] M. Havaux, K. Kloppstech, *Planta* **2001**, *213*, 953–966.
[15] a) S. U. Chon, S. K. Choi, S. Jung, H. G. Jang, B. S. Pyo, S. M. Kim, *Crop Prot.* **2002**, *21*, 1077-1082.; b) M. R. Abenavoli, A. Nicolò, A. Lupini, S. Oliva, A. Sorgonà, *Allelopathy J.* **2008**, *22*, 245-252.
[16] F. Araniti, E. Graña, U. Krasuska, R. Bogatek, M. J. Reigosa, M. R. Abenavoli, A. M. Sánchez-Moreiras, *PloS one* **2016**, *11*, e0160202.
[17] a) I. Casimiro, A. Marchant, R. P. Bhalerao, T. Beeckman, S. Dhooge, R. Swarup, N. Graham, D. Inzé, G. Sandberg, P. J. Casero, M. Bennett, *Plant Cell* **2001**, *13*, 843-852; b) D. R. Lewis, S. Negi, P. Sukumar, G. K. Muday, *Development* **2011**, dev-065102.
[18] E. Graña, T. Sotelo, C. Díaz-Tielas, F. Araniti, U. Krasuska, R. Bogatek, M. J. Reigosa, A. M. Sánchez-Moreiras, *J. Chem. Ecol.* **2013**, *39*, 271-282.
[19] a) H. Buschmann, M. Hauptmanna, D. Niessingc, C.W. Lloydb, A. R. Schäffner, *Plant Cell* **2009**, *21*, 2090-2106.; b) J. C., Sedbrook, D. W. Ehrhardt, S. E. Fisher, W. R. Scheible, C. R. Somerville, *Plant Cell* **2004**, *16*, 1506-1520.
[20] T. Hashimoto, Philos. *Trans. R. Soc. Lond. Ser. B-Biol. Sci.* **2002**, *357*, 799-808.
[21] a) T. Ishida, S. Thitamadee, T. Hashimoto, *J. Plant Res.* **2007**, *120*, 61-70; b) F. Araniti, E. Graña, M. J. Reigosa, A. M. Sánchez-Moreiras, M. R. Abenavoli, *Nat. Prod. Res.* **2013**, *27*, 2297-2303.
[22] M. K. Upadhyaya, L. D. Noodén, *Plant Cell Physiol.* **1978**, *19*, 133-138.
[23] H. Li, S. Yan, L. Zhao, J. Tan, Q. Zhang, F. Gao, P. Wang, H. Hou, L. Li, *BMC Plant Biology* **2014**, *14*, 105.
[24] a) F. Araniti, A. Costas-Gil, L. Cabeiras-Freijanes, A. Lupini, F. Sunseri, M. J. Reigosa, M. R. Abenavoli, A. M. Sánchez-Moreiras, *PloS one* **2018**, *13*, e0208802; b) T. S. Gechev, F. Van Breusegem, J. M. Stone, I. Denev, C. Laloi, *Bioassays* **2006**, *28*, 1091-1101.; c) J. Kacprzyk, P. F. McCabe, A root hair assay to expedite cell death research, in *Plant cell expansion*, Humana Press, New York, NY (USA), **2015**, pp 73-82.
[25] W. Strober, *Curr. Protoc. Immunol.* **1997**, *21*, A-3B.
[26] S. Mukhopadhyay, S. Batra, *Chem. Eur. J.* **2018**, *24*, 14622-14626.
[27] N. Conde, R. SanMartin, M. T. Herrero, E. Domínguez, *Adv. Synth. Catal.* **2016**, *358*, 3283-3292.
[28] M. Barbero, S. Cadamuro, S. Dughera, *Eur. J. Org. Chem.* **2014**, 598-605.
[29] F. Araniti, R. Mancuso, I. Ziccarelli, F. Sunseri, M. R. Abenavoli, B. Gabriele, *Molecules* **2014**, *19*, 8261-8275.
[30] A. R. Wellburn, *J. Plant Physiol.* **1994**, *144*, 307-313.
[31] D. M. Hodges, J. M. DeLong, C. F. Forney, R. K. Prange, *Planta* **1999**, *207*, 604-611.
[32] M. Landi, *Planta* **2017**, *245*, 1067-1067.
[33] a) J. C., Streibig, P. Kudsk, J. E. Jensen, *Pestic. Sci.* **1997**, *53*, 21-28.; b) R. G. Belz, K. Hurle, S. O. Duke, *Toxicol. Med.* **2005**, *3*, 173-211.

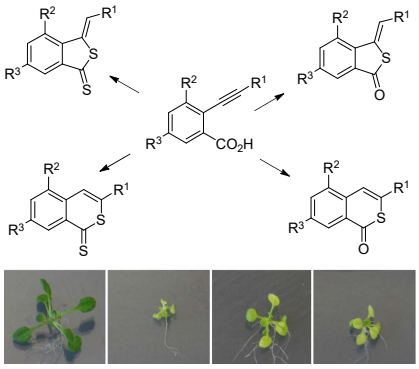
10.1002/cplu.201900316

WILEY-VCH

Entry for the Table of Contents

Microwave-Assisted Synthesis of Sulfurated Heterocycles with Herbicidal Activity: Reaction of 2-Alkynylbenzoic Acids with Lawesson's Reagent

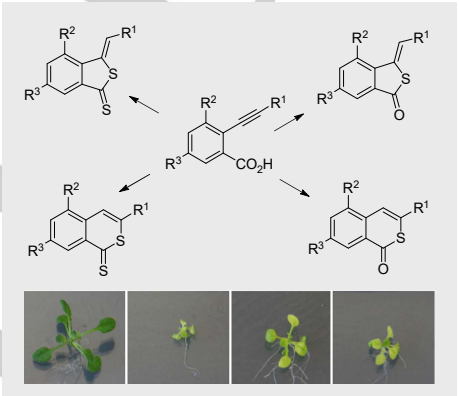
Salvatore V. Giofrè,* Raffaella Mancuso, Fabrizio Araniti,* Roberto Romeo, Daniela Iannazzo, Maria Rosa Abenavoli and Bartolo Gabriele*



Sulfurated heterocycles with herbicidal activity were smoothly synthesized by microwave-assisted monothionation- or dithionation-cycloisomerization of readily available 2-alkynylbenzoic acids using the Lawesson's reagent as thionating agent. Monothionated (benzo[*c*]thiophen-1(3*H*)-ones or 1*H*-isothiochromen-1-ones) or dithionated (benzo[*c*]thiophene-1(3*H*)-thiones or 1*H*-isothiochromene-1-thiones) products were divergently formed depending on the amount of the Lawesson's reagent used and on the substrate substitution pattern. The potential herbicidal activity of these heterocycles was assessed by studying their effect on seedling growth and development of the model species *A. Thaliana*.

FULL PAPER

Sulfurated heterocycles with herbicidal activity were smoothly synthesized by microwave-assisted monothionation- or dithionation-cycloisomerization of readily available 2-alkynylbenzoic acids using the Lawesson's reagent as thionating agent. Monothionated (benzo[*c*]thiophen-1(3*H*)-ones or 1*H*-isothiochromen-1-ones) or dithionated (benzo[*c*]thiophene-1(3*H*)-thiones or 1*H*-isothiochromene-1-thiones) products were divergently formed depending on the amount of the Lawesson's reagent used and on the substrate substitution pattern. The potential herbicidal activity of these heterocycles was assessed by studying their effect on seedling growth and development of the model species *A. Thaliana*.



Salvatore V. Giofrè,[a] Raffaella Mancuso,[b] Fabrizio Araniti,*[c] Roberto Romeo,[a] Daniela Iannazzo,[a] Maria Rosa Abenavoli[c] and Bartolo Gabriele*[b]*

Page No. – Page No.

Microwave-Assisted Synthesis of Sulfurated Heterocycles with Herbicidal Activity: Reaction of 2-Alkynylbenzoic Acids with Lawesson's Reagent