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Synthesis and photosynthetic inhibition activity of substituted 5-(bis-trifluoromethyl)methyl)-2-aminothiazoles

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Abstract

The reaction of 5,5,5-trifluoro-4-trifluoromethyl-pent-3-en-2-one with aminothiocarbonyls yielded aminothiourea precursors which readily cyclised to the corresponding thiazole derivatives, 5-(bis-trifluoromethyl)methyl)-2-aminothiazoles. The inhibitory potency of photosystem II activity of these thiazoles was evaluated using fluorescence measurement techniques. Two of the compounds showed a good activity in comparison with the reference compound DCMU, 3-(3,4-dichlorophenol)-1,1-dimethylurea. Calculation of lipophilicity according to Rekker and Frey, and a corresponding experimental determination are reported.

Keywords: Gem bis-trifluoromethylated molecules; 2-Aminothiazole; PS II inhibitor; Fluorescence; DCMU; Lipophilicity

1. Introduction

Some aminothiazole herbicides inhibit Photosynthetic Electron Transport (PET), cf. Dayan et al. [1], Fig. 1, structure A. The aminothiazoles described are thought to disrupt PET by binding to the D-1 protein of photosystem II (PS II), displacing a plastoquinone (Q_B) from its binding site [2]. Some thiazoles which can act as herbicides have been patented (Fig. 1, structure B) [3]. The thiazole ring is a part of many natural products, such as peptide alkaloids [4], some of which exhibit antibiotic [5–7] and antifungal activities [8]. Natural products containing thiazole rings isolated from marine species show antineoplastic and cytotoxic properties [9]. Cyclopeptides containing thiazole and dihydrothiazole rings also have cytotoxic activity [10–12].

The activity of some pesticides can be improved by substitution of hydrogen with fluorine [13]. As the Van der Waals radii of fluorine and hydrogen are very close (0.135 and 0.12 nm), the bioisosteric replacement of hydrogen by fluorine is frequently used to influence biological properties. This has been particularly useful in pesticides, possibly by improving the penetration of cell membranes or by modifying the mode of binding to a target.

This paper describes the syntheses and herbicidal activities of some new aminothiazoles, containing a bistrifluoromethyl group. An amido moiety seems to be necessary for the activity of PS II herbicides [14]. However, from the patent literature [3] it seemed important to locate a highly fluorinated substituent in position 5 of the thiazole ring. Therefore a combination of structural features of compounds A and B, leading to structure C (Fig. 1) was used to optimize properties.

2. Results and discussion

2.1. Synthesis

5,5,5-Trifluoro-4-trifluoromethyl-pent-3-en-2-one (**1**) was synthesized from hexafluoroacetone and triphenylphosphoranylidene-2-propanone, using the Abele et al. [15] modification of the synthetic method, originally described by Plakova et al. and Pattison [16,17]. A good yield was obtained at room temperature in the presence of hydroquinone (Scheme 1).

Compound **1** is an α,β-unsaturated bistrifluoromethylated olefin of ''inverted activity'' and the presence of two electron-withdrawing CF_3 groups direct nucleophilic attack towards the

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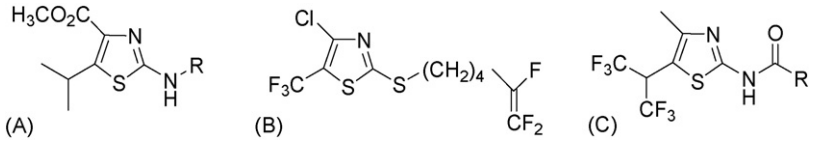
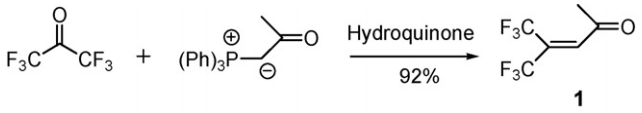


Fig. 1. Known thiazole herbicides A and B and target molecule C.



Scheme 1.

position α to the carbonyl group [18]. A subsequent reaction with an aminothiocarbonyl compound resulted in the formation of an isothiourea derivative, which eliminated water and cyclised forming the desired thiazole derivatives.

Compound **1** reacted with acetyl thiourea in refluxing ethanol yielding *N*-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-acetamide (**2**). This was purified by sublimation and chromatography. Analogously the higher homologue, *N*-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-isopropylamide (**4**), was synthesized from 2-methylpropionyl thiourea (**3**) (prepared from 2-methyl-propionyl chloride and thiourea) and **1** by increasing the reaction time (Scheme 2).

To study the influence of bulky groups, such as chlorophenyl and tertiarybutylphenyl, the corresponding derivatives were synthesized. As these compounds differ only with respect to the substituent on the amino group, the aminothiazole **5** was prepared as a synthon and derivatives were prepared according to Scheme 3.

2.2. Biological properties

2.2.1. Inhibition of photosynthesis

The photosynthesis inhibition activity of compounds **2** and **4**–**9** were first evaluated using unicellular algae *Chlamydomonas reinhardtii*, and subsequently using *Arabidopsis* plants. Tests were based on fluorescence measurements, as fluorescence is inversely related to photosynthetic activity [19]. The inhibition is calculated relative to the reference inhibitor, 3-(3,4-dichlorophenol)-1,1-dimethylurea (DCMU). The procedure is reported in the experimental section. Results for the *Chlamydomonas* test are summarized in Table 1.

Table 1
Compounds tested and their relative inhibition activity on photosynthesis for the unicellular algae *Chlamydomonas reinhardtii*

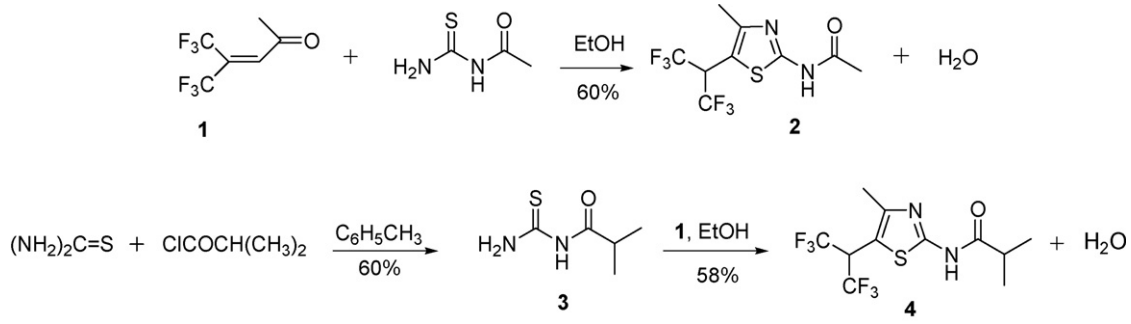
Compound	Maximal inhibition (r.u.)[a]	Concentration (μM)	IC_{50}[b] (μM)
2	1.00	10	0.85
4	1.00	10	1.82
5	0.59	1.0×10^3	–
6	0.45	1.0×10^6	–
7	0.70	3.0×10^6	–
8	0.73	1.0×10^4	–
9	0.34	3.0×10^3	–
DCMU	1.00	1.0	0.12

(–) Undetermined, low inhibition at strong concentrations.
[a] Inhibition is in relative units. See Section 3 for the calculation method.
[b] Concentration of inhibitor required for 50% inhibition.

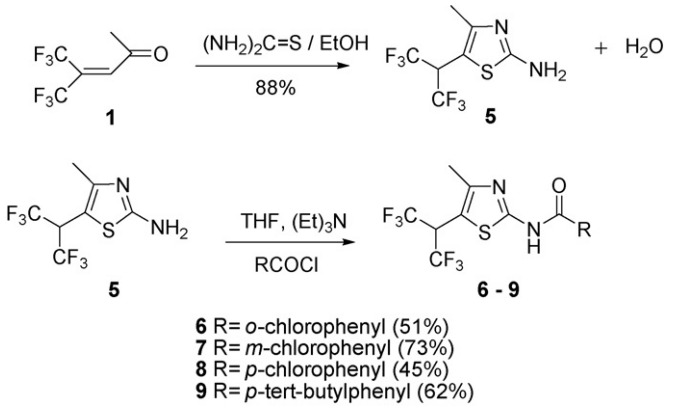
Even at low concentration (10 μM), compounds **2** and **4** showed 100% inhibition. This means, that in the presence of these compounds, there is no photosynthetic activity and all the light energy absorbed is reemitted as fluorescence (and heat). The IC_{50} values obtained for compounds **2** and **4** (0.85 and 1.82 μM) are good when compared with DCMU (0.12 μM), which is one of the best PS II inhibitors known.

The compounds with a chlorophenyl ring as a substituent (**6**–**8**) showed weak to medium activity, which increased from the *ortho* to the *para* position. These compounds were not further investigated, just as compounds **5** and **9**.

The activity of compounds **2** and **4** have also been evaluated using *Arabidopsis* plants, by the method of Barbagallo et al. [20]. Chlorophyll fluorescence imaging can effectively and rapidly identify perturbations of leaf metabolism, well before the onset of any visual effects of leaf morphology or plant growth. Solutions of inhibitors can be directly sprayed onto the leaf and the fluorescence recorded photographically. Graphs



Scheme 2.



Scheme 3.

obtained by this method are in accordance with those obtained from *Chlamydomonas*.

2.2.2. Lipophilicity

To try and explain the variations in activity, the lipophilicity of the compounds tested was calculated and measured. Based on general considerations, it has to be assumed that in comparison to its hydrogen analogue, the presence of fluorine in a molecule does not necessarily increase its lipophilicity [21–23]. On the other hand the variation of substituents on the amino group influences the lipophilicity which is represented by corresponding log *P* values.

Those were calculated using the Rekker and Mannhold method [24]. Since Bate-Smith and Westall observed the correlation between chromatographic properties and log *P* [25], measurements were made using reverse phase thin layer chromatography, according to Frey et al. [26] and Dross et al. [27] (giving R_M values, cf. chapter 3.2.3).

We defer only the values obtained for the active compounds **2** and **4** (Table 2). Their lipophilicity is in a median fork in comparison with the other compounds prepared (**5**-**9**, $2.4 < R_M < 7$).

3. Experimental

3.1. Chemistry

NMR spectra were recorded on Brucker AC 300 (^{1}H 300.13; ^{13}C 75.47; ^{19}F 282.40 MHz) instrument. Chemical shift values are given in ppm relative to tetramethylsilane. High-resolution mass spectra (HRMS) were recorded on JEOL JMS SX 120A instrument. Melting points were determined on a Stuart Scientific apparatus and are uncorrected.

3.1.1. Synthesis of N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-acetamide (2)

A mixture of acethyl thiourea (1.18 g, 10 mmol) and 5,5,5-trifluoro-4-trifluoromethyl-pent-3-en-2-one **1** (1.97 g, 9.5 mmol) in ethanol (30 mL) was refluxed with stirring for 4 d. Ethanol was evaporated and the crude reaction product was purified firstly by column chomatography (eluent: pentane/diethylether, 10:90), then by sublimation, to give 1.76 g (60%) of **2** as a white solid, mp: 185–189 °C.

^{1}H NMR ($CDCl_3$) : δ 2.20 (s, 3H, C(=O)CH_3); 2.28 (s, 3H, CH_3 thiazole); 4.3 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.89 Hz); ^{13}C NMR ($CDCl_3$) : δ 15.06 (s, 1C, CH_3 thiazole); 23.12 (s, 1C, CO$\underline{C}H_3$); 47.72 (h, 1C, $C(CF_3)_2$, J_{CF} = 31.3 Hz); 109.03 (s, 1C, C_5 thiazole); 122.38 (q, 2C, $(CF_3)_2$, J_{CF} = 271.7 Hz) ; 148.9 (C=O); 158.9 (s, 1C, C_4 thiazole); 167.79 (s, 1C, C_2 thiazole); ^{19}F NMR ($CDCl_3$) : δ –66,3 (6 F, d, J_{HF} = 5.6 Hz). HR-MS: 307.0356 ($C_9H_9ON_2F_6S^+$; calc. 307.0340).

3.1.2. Synthesis of 2-methylpropionyl thiourea (3)

Thiourea (2 g, 26.3 mmol) was dissolved in toluene (25 mL). 2-Methylpropionyl chloride (2.74 mL, 2.79 g, 26.3 mmol) was added and the reaction mixture was stirred and refluxed for 2 h. The reaction flask was connected to a water trap in order to collect HCl vapors. The crude product crystallized partially on cooling at room temperature. The solvent was evaporated in vacuo and the residue was recrystallized from ethanol to give 2.3 g (60%) of **3** as a white solid, mp: 114–116 °C (lit. 114.5 °C [28]).

^{1}H NMR ($CDCl_3$) : δ 1.24 (d, 2 × 3H, C(=O)CH($C\underline{H}_3$)$_2$, J_{HH} = 6 Hz); 2.68 (h, 1H, $C\underline{H}(CH_3)_2$, J_{HH} = 6.93 Hz).

3.1.3. Synthesis of N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-isopropylamide (4)

A mixture of 2-methylpropionyl thiourea **3** (2.2 g, 15 mmol) and 5,5,5-trifluoro-4-trifluoromethyl-pent-3-en-2-one **1** (3.8 g, 18.4 mmol) in ethanol (30 mL) was stirred at reflux for 2 d. Ethanol was evaporated and the crude reaction product was purified by column chromatography (eluent: pentane/diethylether, 30:70) to give 2.9 g (57%) of **4** as a white solid, mp: 136–140 °C.

^{1}H NMR ($CDCl_3$) : δ 1.22 (d, 2 × 3H, CH($C\underline{H}_3$)$_2$, J_{HH} = 6.93 Hz); 2.28 (s, 3H, CH_3 thiazole); 2.57 (h, 1H, $C\underline{H}(CH_3)_2$, J_{HH} = 6.93 Hz); 4.31 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.89 Hz); 9.5 (br., 1H, NH); ^{13}C NMR ($CDCl_3$) : δ 13.91 (s, 1C, CH_3 thiazole); 16.5 (s, 1C, CH_3) ; 16.74 (s, 1C, CH_3); 33.07 (s, 1C, $C(CH_3)_2$); 45.38 (h, 1C, $C(CF_3)_2$, J_{CF} = 30.94 Hz); 106.72 (s, 1C, C_5 thiazole) ; 120.15 (q, 2C, $(CF_3)_2$, J_{CF} = 282.3 Hz); 146.45 (C=O) ; 157.0 (s, 1C, C_4 thiazole) ; 172.91 (s, 1C, C_2 thiazole); ^{19}F NMR ($CDCl_3$) : δ –66,3 (6 F, d, J_{HF} = 8.4 Hz). HR-MS: 335.0634 ($C_{11}H_{13}ON_2F_6S^+$; calc. 335.0653).

3.1.4. Synthesis of 4-methyl-5-(bis trifluoromethyl)methyl-2-aminothiazole (5)

A mixture of thiourea (2.95 g, 38.8 mmol) and 5,5,5-trifluoro-4-trifluoromethyl-pent-3-en-2-one **1** (4 g, 19.4 mmol)

Table 2
Calculated log *P* and R_M values for compounds **2** and **4**

	Compounds	
	2	**4**
log *P*	1.0	2.04
R_M	2.84	3.59

in ethanol (25 mL) was stirred at reflux for 2 h. A white precipitate appeared when cooling at room temperature (remaining thiourea). The solution was filtered and the solvent was removed in vacuo. The residue was washed with water, and then extracted three times with diethylether. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The yellowish solid obtained was purified by sublimation to give 4.5 g (87%) of **5** as a white solid, mp : 104–108 °C (lit. : 106–107 °C [18]).

^{1}H NMR ($CDCl_3$): δ 2.2 (s, 3H, CH_3 thiazole); 4.28 (h, 1H, 7-CH, J_{HF} = 7.9 Hz); 5.0 (br., 2H, NH_2); ^{13}C NMR ($CDCl_3$) : δ 14.95 (s, 1C, CH_3 thiazole); 47.68 (hept, 1C, $C(CF_3)_2$, J_{CH} = 30.9 Hz); 102.79 (s, 1C, C_5 thiazole); 122.52 (2 × q, 2C, $(CF_3)_2$, J_{CF} = 284.5 Hz); 150.86 (s, 1C, C_4 thiazole); 168.13 (s, 1C, C_2 thiazole); ^{19}F NMR ($CDCl_3$) : δ –66,7 (6 F, d, J_{HF} = 8,5 Hz). HR-MS: 265.0222 ($C_7H_7N_2F_6S^+$; calc. 265.0234).

3.1.5. Synthesis of 2-chloro-N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-benzamide (6)

4-Methyl-5-(bis trifluoromthyl)methyl-2 aminothiazole **5** (0.9 g, 3.4 mmol) was dissolved in THF (25 mL). Triethylamine (0.52 mL, 0.38 g, 3.7 mmol) was added and the reaction mixture was stirred. The 2-chlorobenzoyl chloride (0.65 g, 0.5 mL, 3.7 mmol) was added drop by drop, resulting in a turbid suspension. After stirring for 0.5 h at room temperature, the precipitate ($HCl.Et_3N$) was filtered and the solvent was removed in vacuo. The residue was washed with water, then extracted by diethylether. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The orange residue was purified by sublimation to give 0.7 g (51%) of **6** as a white solid, mp: 120–130 °C.

^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3H, CH_3 thiazole); 4.33 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.9 Hz); 7.22-7.48 (m, 2H, arom.); 7.78 (dd, 1H, J_{HH} = 0.7 Hz, J_{HH} = 7.3 Hz); 7.89 (d, 1H, J_{HH} = 7.3 Hz); ^{13}C NMR ($CDCl_3$) : δ 14.37 (s, 1C, CH_3 thiazole); 47.7 (h, 1C, $C(CF_3)_2$, J_{CF} = 31.7 Hz); 109.23 (s, 1C, C_5 thiazole); 127.39 (s, 1C, arom.); 130.81 (s, 1C, arom.); 130.84 (s, 1C, arom.); 131.7 (s, 1C); 132.9 (s, 1C, arom.); 149.29 (s, 1C); 159.34 (s, 1C); 164.12 (s, 1C); 170.22 (s, 1C); ^{19}F ($CDCl_3$) : δ –66,2 (6 F, d, J_{HF} = 8,5 Hz). HR-MS: 403.0101 ($C_{14}H_{10}ON_2ClF_6S^+$; calc. 403.0107).

3.1.6. Synthesis of 3-chloro-N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-benzamide (7)

4-Methyl-5-(bis trifluoromethyl)methyl-2-aminothiazole **5** (0.62 g, 2.3 mmol) was dissolved in THF (15 mL). Triethylamine (0.36 mL, 0.26 g, 2.6 mmol) was added and the reaction mixture was stirred. The 3-chlorobenzoyl chloride (0,33 mL, 0,45 g, 2,6 mmol) was added drorwise, resulting in a turbid suspension. After stirring 0.5 h at room temperature, the precipitate ($HCl.Et_3N$) was filtered off and the solvent was removed in vacuo. The residue was washed with water, and then extracted with diethylether. The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. The orange residue was purified by sublimation to give 0.69 g (73%) of **7** as a white solid, mp: 201–205 °C.

^{1}H NMR (DMSO) : δ 2.40 (s, 3H, CH_3 thiazole); 5.9 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.5 Hz); 7.58 (d, 1H, arom., J_{HH} = 7.9 Hz); 7.71 (dd, 1H, arom., J_{HH} = 1.06 Hz, J_{HH} = 7.24 Hz); 8.02 (d, 1H, arom., J_{HH} = 7.78 Hz); 8.14 (s, 1H, arom.); ^{13}C NMR ($CDCl_3$) : δ 14.78 (s, 1C, CH_3 thiazole); 45.43 (h, 1C, $C(CF_3)_2$, J_{CH} = 30.2 Hz); 107.45 (s, 1C, C_5 thiazole); 123.01 (2 × q, 2C, $(CF_3)_2$, J_{CF} = 280.75 Hz); 126.88 (s, 1C, arom.); 127.92 (s, 1C, arom.); 130.56 (s, 1C, arom.); 132.5 (s, 1C, arom.); 133.4 (s, 1C); 133.7 (s, 1C); 159.8 (s, 1C); 164.33 (s, 1C); ^{19}F NMR ($CDCl_3$) : δ –65.6 (6 F, d, $^3J_{HF}$ = 8,5 Hz). HR-MS: 403.0100 ($C_{14}H_{10}ON_2ClF_6S^+$; calc. 403.0107).

3.1.7. Synthesis of 4-chloro-N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-benzamide (8)

4-Methyl-5-(bis trifluoromethyl)methyl-2-aminothiazole **5** (1.3 g, 4.9 mmol) was dissolved in THF (25 mL). Triethylamine (0.75 mL, 0.54 g, 5.4 mmol) was added and the reaction mixture was stirred. 4-Chlorobenzoyl chloride (0.69 mL, 0.95 g, 5.4 mmol) was added dropwise, resulting in a turbid suspension. After stirring 0.5 h at room temperature, the precipitate ($HCl.Et_3N$) was filtered off and the solvent was removed in vacuo. The residue was washed with water, and then extracted with diethylether. The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The brown-orange oil was purified by column chromatography (eluent: light petroleum/diethylether, 70:30) to give 0.91 g (46%) of **8** as a white solid, mp: 145–150 °C.

^{1}H NMR ($CDCl_3$) : δ 1.99 (s, 3H, CH_3 thiazole); 4.3 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.84 Hz); 7.37 (d, 1H, arom., J_{HH} = 8.57 Hz); 7.82 (d, 1H, arom., J_{HH} = 8.28 Hz); 11 (1H, br., NH); ^{13}C NMR ($CDCl_3$) : δ 13.53 (s, 1C, CH_3 thiazole); 47.13 (h, 1C, $CH(CF_3)_2$, J_{CH} = 30.187 Hz) ; 108.26 (s, 1C, C_5 thiazole); 121.17 (2 × q, 2C, $(CF_3)_2$, J_{CF} = 280.0 Hz); 127.12 (s, 1C, arom.); 128.14 (s, 1C, arom.); 130.22 (s, 1C, arom.); 131.69 (s, 1C, arom.); 138.74 (s, 1C); 147.41 (s, 1C); 159.16 (s, 1C); 163.39 (s, 1C); 169.86 (s, 1C); ^{19}F NMR ($CDCl_3$): δ –66,3 (6 F, d, J_{HF} = 8,5 Hz). HR-MS: 403.0090 ($C_{14}H_{10}ON_2ClF_6S^+$; calc. 403.0107).

3.1.8. Synthesis of 4-tert-butyl-N-(5-(2,2,2-trifluoro-1-trifluoromethyl-ethyl)-4-methyl-thiazol-2-yl)-benzamide (9)

4-Methyl-5-(bis trifluoromethyl)methyl-2-aminothiazole **5** (1.3 g, 4.9 mmol) was dissolved in THF (10 mL). Triethylamine (0.9 g, 9 mmol) and 4-tertiobutylbenzoyl chloride (1.06 g, 5.4 mmol) in THF (3 mL) were added and the mixture was warmed to reflux for 24 h. After evaporating in vacuo, the brown oil residue was purified by colomn chromatography (eluent: light petroleum/diethylether, 60:40). The yellowish oil obtained crystallized to give 1.3 g (62%) of **9** as a yellowish solid, mp: 135–140 °C.

^{1}H NMR ($CDCl_3$) : δ 1.26 (s, 3H, $C(CH_3)_3$); 1.27 (s, 3H, $C(CH_3)_3$); 1.28 (s, 3H, $C(\underline{CH}_3)_3$); 2.01 (s, 3H, CH_3 thiazole); 4.3 (h, 1H, $CH(CF_3)_2$, J_{HF} = 7.89 Hz); 7.42 (d, 2H, arom., J_{HH} = 8.52 Hz); 7.8 (d, 2H, arom., J_{HH} = 8.56 Hz); ^{13}C NMR ($CDCl_3$) : δ 14.45 (s, 1C, CH_3 thiazole); 31.01 (s, 3C, 3 × CH_3); 35.17 (s, 1C, $C(CH_3)_3$); 47.69 (h, 1C, $C(CF_3)_2$, J_{CF} = 30.9 Hz); 109.2 (s, 1C, C_5 thiazole); 126.01 (s, 1C, arom.) ; 127.74 (s, 1C, arom.) ; 128.61 (s, 1C, arom.) ; 148.67 (C═O) ; 157.24 (s, 1C,

C_4 thiazole) ; 160.16 (s, 1C, arom.) ; 165.31 (C_2 thiazole); ^{19}F NMR ($CDCl_3$): δ –66,3 (6 F, d, J_{HF} = 8,5 Hz). HR-MS: 425.1151 ($C_{18}H_{19}ON_2F_6S^+$; calc. 425.1122).

3.2. Biological test methods

3.2.1. Chlamydomonas tests

3.2.1.1. Strains and culture conditions. Chlamydomonas reinhardtii wild-type (from strain 137 C) were grown at 24 °C in tris-acetate-phosphate (TAP)-supplemented medium [29] under 6 μE (E = mol photon/m^2/s) white light. The cells were harvested during exponential growth ($\sim 2 \times 10^6$ cells/mL), centrifuged (at 20 °C, for 5 min, 4000 RP speed) and resuspended in minimal medium [29].

3.2.1.2. Fluorescence measurements. Fluorescence measurements were performed at room temperature on a home-built fluorimeter: samples were excited using a light source at 590 nm with ~60 μE intensity. The fluorescence response was detected in the far red region of the spectrum.

3.2.1.3. Samples. Compounds were tested at different concentrations (100 to 0.1 μM). Stock solutions with a concentration of 0.1 M were obtained by dissolving the tested compounds in ethanol. Solutions of different concentrations were obtained by diluting the stock solution with water. Final aliquots tested were obtained by mixing the solution containing the inhibitor (at proper concentration) with 1 mL algae suspension.

3.2.1.4. Test progress and inhibition calculations. The fluorescence measurement provides F_0, F_m and F_{**} values. The first fluorescence measure is one of a sample of pure algae (''white'') and algae with DCMU (''white DCMU''). Then, primary to the mixture inhibitor/algae test (providing F_m inhibitor) and mixture DCMU/algae test (providing F_m DCMU), for each concentration of inhibitor, the pure algae solution was first tested in order to acquire a ''control result'' (providing F_0 control). The inhibition value results from the calculation of:

$$\text{inhibition}(r.u.) = \frac{X - Y}{1 - Y}$$

$$X = \frac{F_m(\text{inhibition}) - F_0(\text{control})}{F_m(\text{DCMU}) - F_0(\text{control})}$$

$$Y = \frac{F_{**}(\text{''white''}) - F_0(\text{''white''})}{F_m(\text{''whiteDCMU''}) - F_0(\text{''white''})}$$

3.2.2. Arabidopsis tests

Mixtures of 3 mL of 1 mM solution of compound **2**, **4** and DCMU, with 0.1% (in volume) of a solution of Tween 20 were prepared respectively. They were sprayed on leaves of three different plants. A reference plant was spayed with a water mixture containing 1% (in volume) of ethanol and 0.1% (in volume) of Twin 20. After four hours incubation, the kinetics of fluorescence were measured with a home made fluorimeter camera.

3.2.3. Lipophilicity measures

Chromatographical evaluation of lipophilicity was realized using commercially available TLC aluminium sheets (size 20 cm × 20 cm, silicagel RP-18 F_{254s}; MERCK). 3 μL of a methanolic solution of the test compounds were applied to the plate. The developing solvent was 100/0, 90/10, 80/20, 75/25, 70/30 and 60/40 mixtures of MeOH/H_2O. Measuring the distance of the substance maximum from front and start afforded R_f values. R_M values have been calculated according to Bate-Smith and Westall [25]:

$$R_M = \log[(1/R_F) - 1]$$

A linear correlation plot of % of MeOH versus R_M gave R_M values for 100% H_2O.

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