SUPPLEMENTARY MATERIAL

Synthesis of some polyphenolics from contents of onion skin and discovery of the promoter effect on barley germination

Şevket Hakan ÜNGÖREN,[a,*] İbrahim Evren KIBRIZ,[a] Halil Erhan EROĞLU[b]

[a]Department of Chemistry, Faculty of Arts and Sciences, Yozgat Bozok University, 66200 Yozgat, Tükiye

[b]Department of Biology, Faculty of Arts and Sciences, Yozgat Bozok University, 66200, Yozgat, Türkiye

Abstract: Facile synthesis and characterization of three natural compounds and their two synthetic analogues based on onion skin content were performed. Both OSE and 2,4,6-trihydroxyphenylglyoxylic acid was induced effect on cell proliferation during barley germination with a difference of approximately %4 compared to the control group.

Keywords: Quercetin, phenylglyoxylate, onion skin, barley germination, growth regulation, auxin

*Corresponding author

E-mail address: shakan.ungoren@bozok.edu.tr

Content

3. Experimental part 3
3.1 Materials and methods 3
3.2 Extraction of onion skin sample 3
3.3 Cytogenetic procedure 3
Figure S1. The meristematic cells after exposure to compound 2. 4
Figure S2. The mitotic index rates in barley cells after exposure to 2 concentrations. 4
3.4 4,6-Dihydroxybenzofuran-2,3-dione (1) 5
Scheme S1. ^{1}H NMR spectra of 1. 5
Scheme S2. ^{13}C NMR spectra of 1. 6
3.5 2,4,6-Trihydroxyphenylglyoxylic acid (2) 6
Scheme S3. ^{1}H NMR spectra of 2. 7
Scheme S4. ^{13}C NMR spectra of 2. 7
3.6 Methyl 2,4,6-trihydroxyphenylglyoxylate (3) 8
Scheme S5. ^{1}H NMR spectra of 3. 8
Scheme S6. ^{13}C NMR spectra of 3. 9
3.7 Ethyl 2,4,6-trihydroxyphenylglyoxylate (4) 9
Scheme S7. ^{1}H NMR spectra of 4. 10
Scheme S8. ^{13}C NMR spectra of 4. 10
3.8 *i*-Propyl 2,4,6-trihydroxyphenylglyoxylate (5) 11
Scheme S9. ^{1}H NMR spectra of 5. 11
Scheme S10. ^{13}C NMR spectra of 5. 12
3.9 The meristematic cells images after exposure to 1 mL water extract prepared from 5g/50mL onion skin extract: 12
3.10 The meristematic cells images after exposure to 1 mL solution prepared from 1 mg/mL of compound 2: 14
3.11 The meristematic cells images after exposure to 1 mL solution prepared from 2 mg/mL of compound 2: 15
3.12 The meristematic cells images after exposure to 1 mL solution prepared from 3 mg/mL of compound 2: 17
3.13 Control experiment; the meristematic cells images after exposure to 1 mL distilled water 18

3. Experimental part

3.1 Materials and methods

The reactions were carried out under atmosphere. The new products were purified with crystallizations by proper solvents. All experiments were monitored by TLC using DC Alufolien Kieselgel 60F 254 Merck and Camag TLC lam (254/366 nm). All solvents and reagents were purchased from Merck. The apparatus used in the experiments: Bruker Advance 400 in DMSO-d_6 by using D_2O as an internal (^1H (300.13 MHz) and ^{13}C (75.47 MHz) NMR spectra); Agilent Technologies 6224 TOF LC/MS using ESI ionization (HR MS spectra); Perkin Elmer Spectrum Two Model FT-IR (IR spectra); Electrothermal 9200 (melting point).

3.2 Extraction of onion skin sample

5.0 g of brown onion skin cut into small pieces were added to 50 mL of distilled hot water. After stirring at room conditions for 3 days, it was filtered through a filter paper.

3.3 Cytogenetic procedure

Barley (*Hordeum vulgare* L.) seeds were germinated in petri dishes by the 1 mL extract of onion skin and different concentrations of compound **2** (1, 2, and 3 mg/mL) at room temperature for 3 days. The roots forming the control group were germinated with water. Then, the root tips were fixed in fixative solution (ethanol:acetic acid, 3:1 v/v) for 24 h, were transferred into 70% ethyl alcohol and stored at 4 ºC until analysis. For the analysis, the roots were hydrolysed in 1N HCl at 60 ºC for 8 min and washed with distilled water. Finally, the root tips were stained in aceto-orcein for 2h and squashed in 45% acetic acid.

In microscopic analysis, ten slides for each concentration were observed under research light microscope (Olympus BX53, Japan) and photographed by digital camera (Olympus DP72, Japan). About 2000 cells were analysed for each concentration in the evaluation of mitotic index (MI). MI was calculated as follows.

MI (%) = (Total number of cells in mitosis / Total number of cells) × 100

The data were calculated by one-way analysis of variance (ANOVA) and differences among groups were determined by the Tukey test ($p < 0.05$) in MS-Excel software.

The meristematic cells after exposure to compound 2. Dividing cells in different stages of mitosis are highlighted with arrows as shown Figure S1.



Figure S1. The meristematic cells after exposure to compound 2.

The mitotic index rates in barley cells after exposure to 2 concentrations. Statistically significant differences are highlighted with letters, Figure S2.

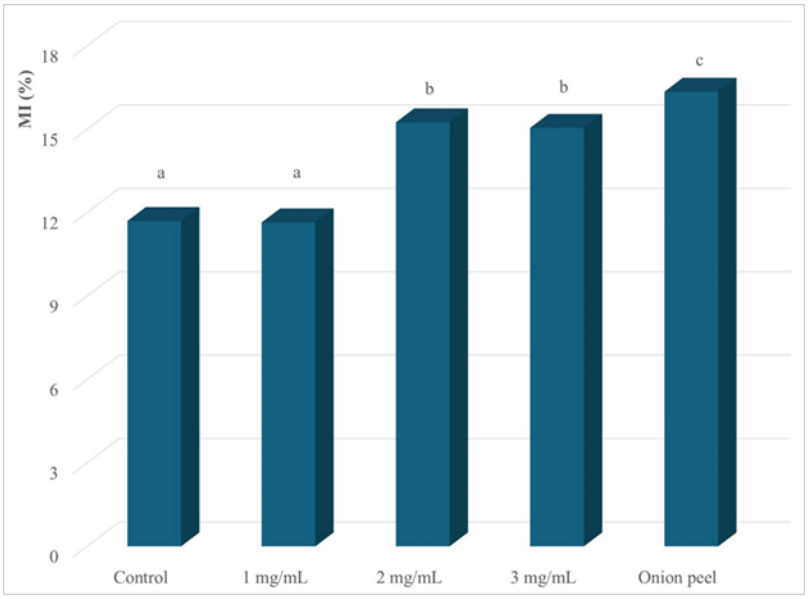
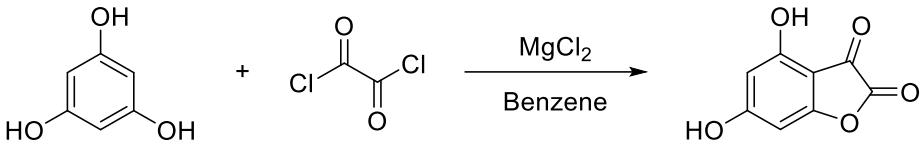
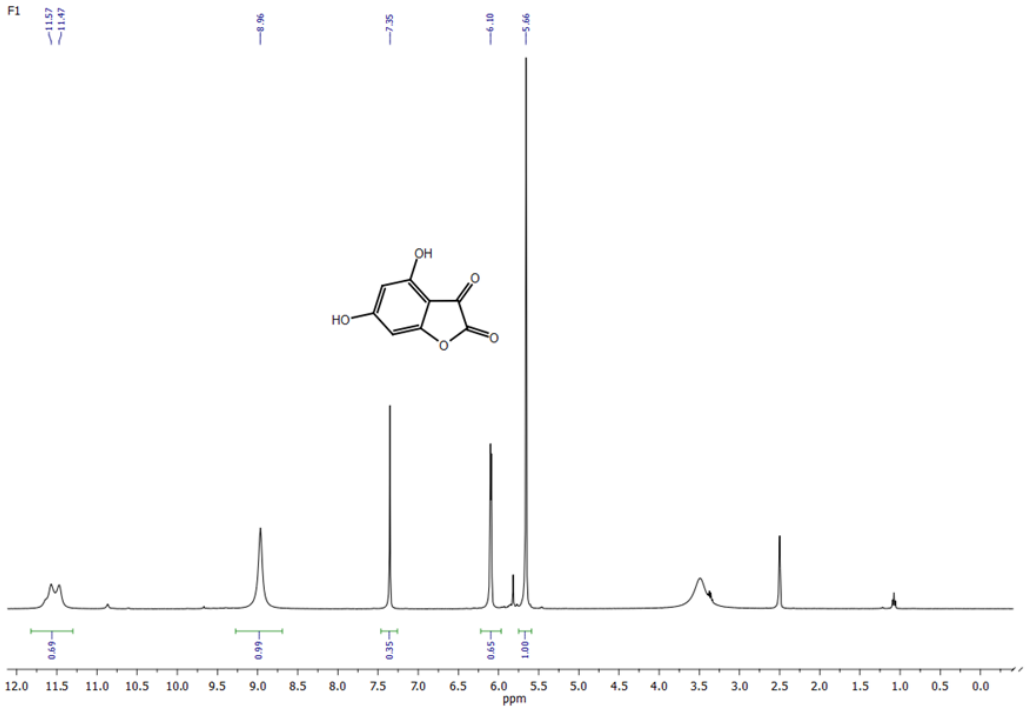


Figure S2. The mitotic index rates in barley cells after exposure to 2 concentrations.

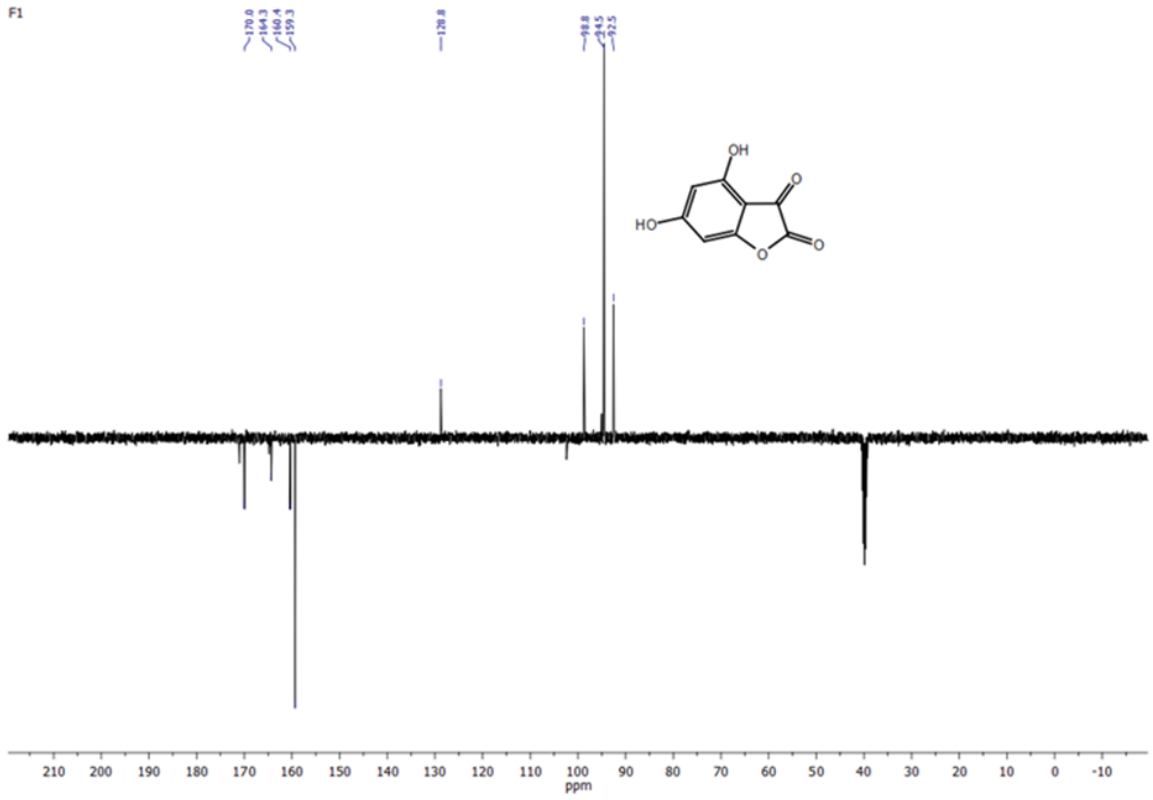
3.4 4,6-Dihydroxybenzofuran-2,3-dione (1)



To a suspension of phloroglucinol (0, 378 g, 3 mmol) and 0.095 g $MgCl_2$ as catalyst in 50 mL benzene was added by dropwise the solution of oxalyl chloride (0.381 g, 3 mmol) in 5 mL benzene and vigorously stirred for 1 h. in room temperature. Then, the white suspension was refluxed additional 2 h. Meanwhile, the suspension turns yellow. Lactone (**1**) was filtered and recrystallized from toluene-ethyl acetate mixture (5:1) as yellow solid. M.p. 197-198 ºC (dec.), yield 0,410g, 76%. ^{1}H NMR (400 MHz, DMSO, δ, ppm): 11.57-8.96 (s, 2H, 2xOH), 7.35-5.66 (m, 2H, Ar-H). ^{13}C NMR (101 MHz, DMSO, δ, ppm): 170.0, 169.5, 164.8,164.3, 160.4, 159.4, 128.8, 102.4, 98.8, 95.0, 94.5, 92.5. FTIR (ATR, cm^{-1}): 3308, 3170 (OH), 1801, 1701 (C=O). HRMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_8H_5O_5^+$: 181.0131; found: 181.0133.

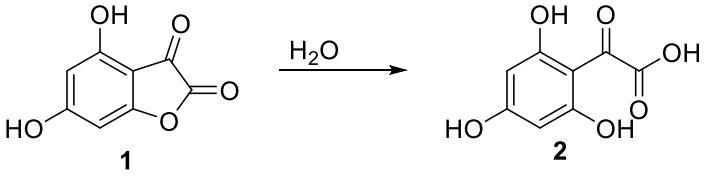


Scheme S1. ^{1}H NMR spectra of 1.

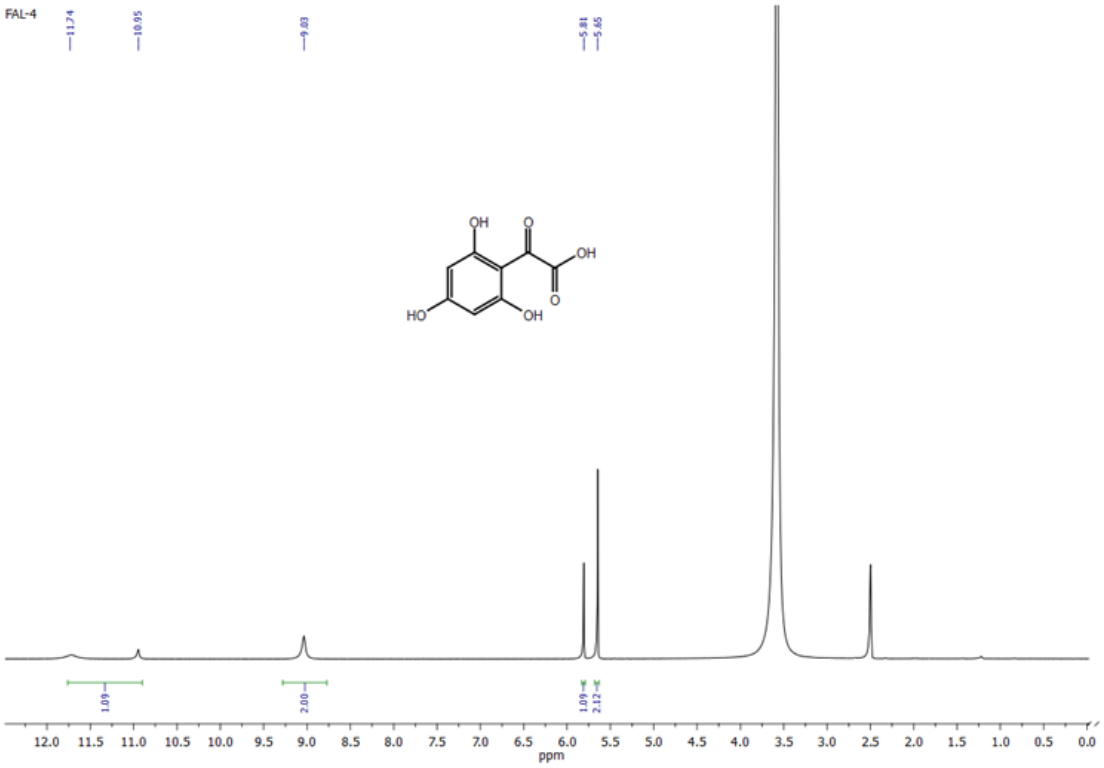


Scheme S2. ^{13}C NMR spectra of 1.

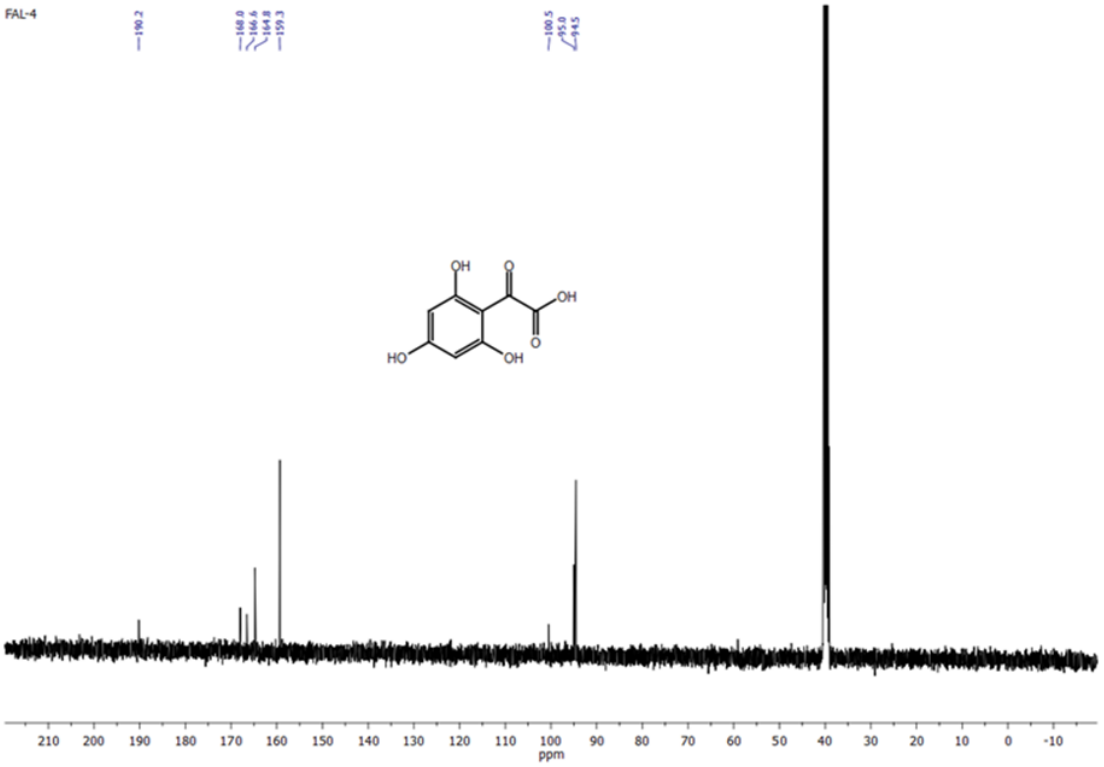
3.5 2,4,6-Trihydroxyphenylglyoxylic acid (2)



1 mL hot H_2O was added to **1** (0.180 g, 1 mmol) and stirred for 1 min. Then, the solvent was evaporated to dryness at open air. The pure dull yellow solid (**2**) was dried on P_2O_5. M.p. 141-143 ^oC, yield 0,190 g, 96%. ^1H NMR (400 MHz, DMSO, δ, ppm): 11.74, 10.95, 9.03, 5.81 (s, 4H, 4xOH), 5.65 (s, 2H, ArCH). ^{13}C NMR (101 MHz, DMSO, δ, ppm): 190.2, 159.3 (C=O), 168.0, 166.6, 164.8 (ArC-O), 100.5 (ArC-CO), 95.0, 94.5 (=CH). FTIR (ATR, cm^{-1}): 3465, 3334, 3176 (OH), 1631 (C=O). HRMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_8H_7O_6^+$: 199.0237; found: 199.0224.

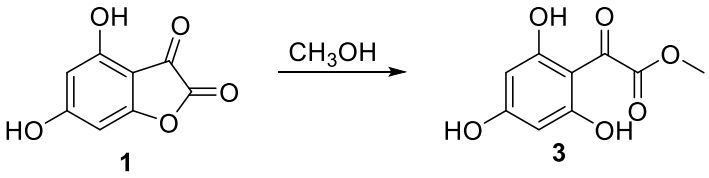


Scheme S3. ^{1}H NMR spectra of 2.

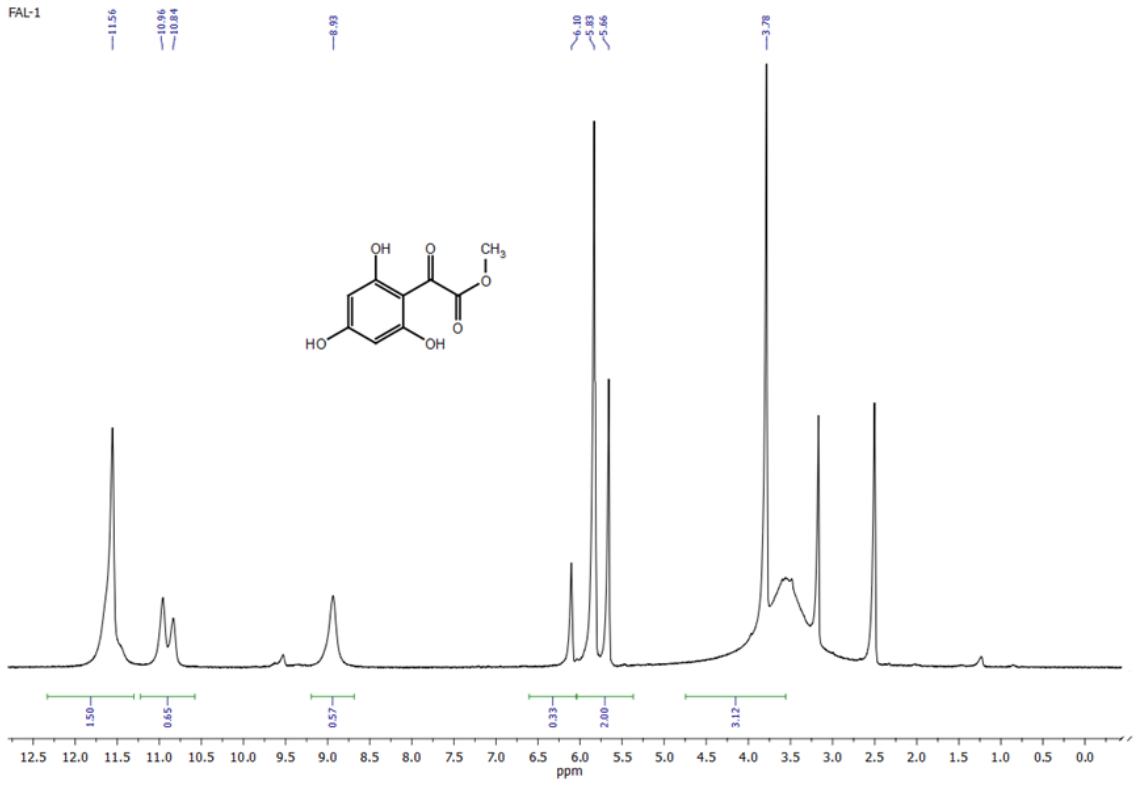


Scheme S4. ^{13}C NMR spectra of 2.

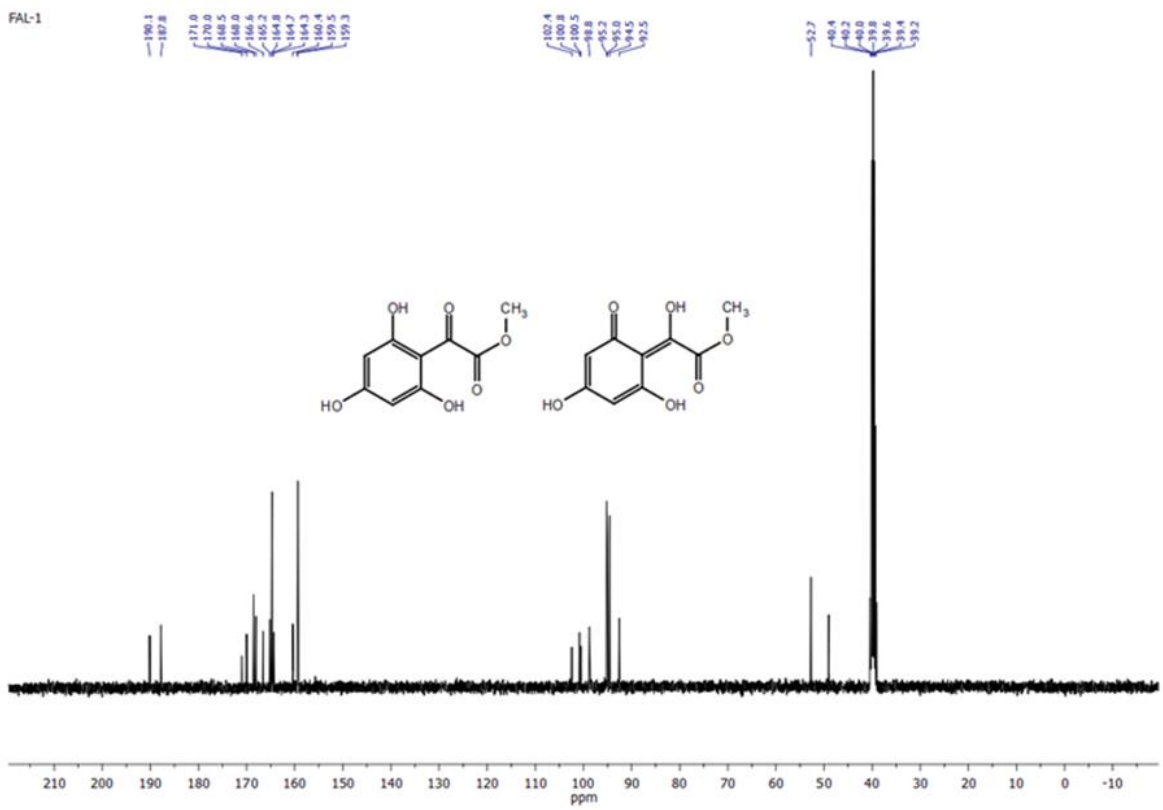
3.6 Methyl 2,4,6-trihydroxyphenylglyoxylate (3)



This compound was prepared from 1 mmol of **1** with 1 mL MeOH according to preparation procedure of **2**. Dull yellow solid, m.p. 95-97 ^{o}C, yield 0,200 g, 93%. ^{1}H NMR (400 MHz, DMSO, δ, ppm): 11.56-6.10 (s, 3H, 3xOH), 5.83-5.66 (s, 2H, =CH), 3.78 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, DMSO, δ, ppm): 190.1, 187.8 (keto-enol form's C=O), 171.0, 170.0, 168.5, 168.0, 166.6, 165.2, 164.8, 164,7, 164.3, 160,4, 159.5, 159.3, 102.4, 100.8, 100.5, 98.8, 95.2, 95.0, 94.5, 92.5 (C=O and C=C), 52.7, 49.0 (two tautometic form's OCH_3). FTIR (ATR, cm^{-1}): 3467, 3221 (OH), 1726, 1639 (C=O). HRMS (ESI): *m/z* $[M + H]^+$ calcd. for $C_9H_9O_6^+$: 213.0394; found: 213.0395.

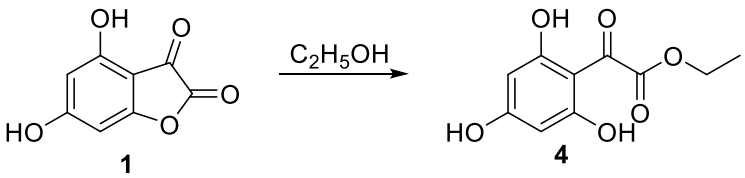


Scheme S5. ^{1}H NMR spectra of 3.

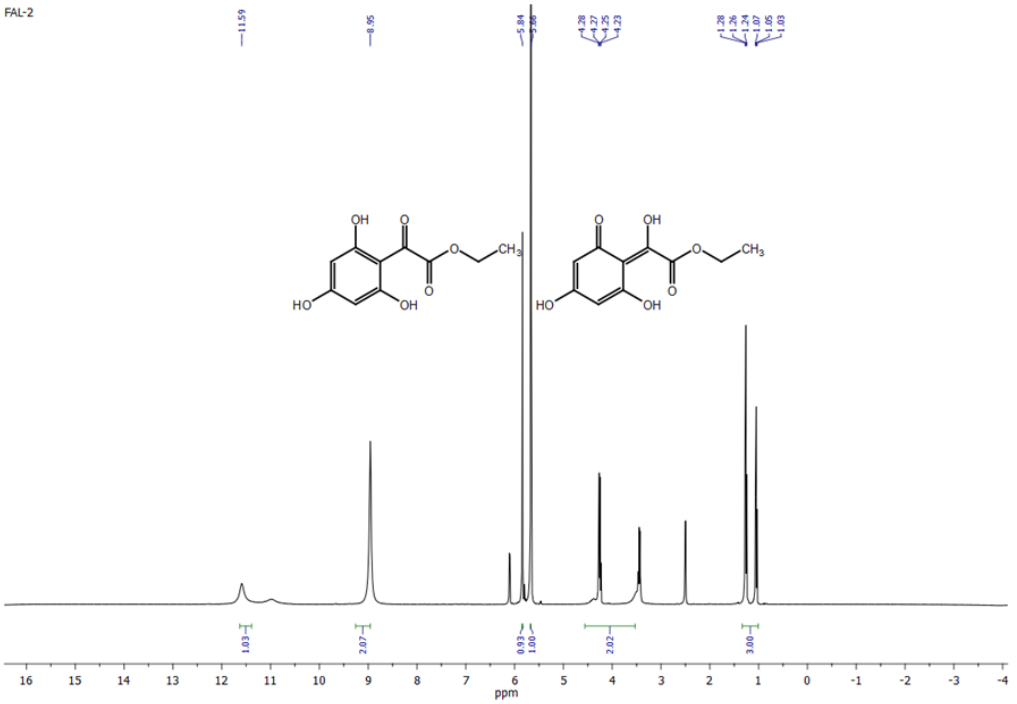


Scheme S6. ^{13}C NMR spectra of 3.

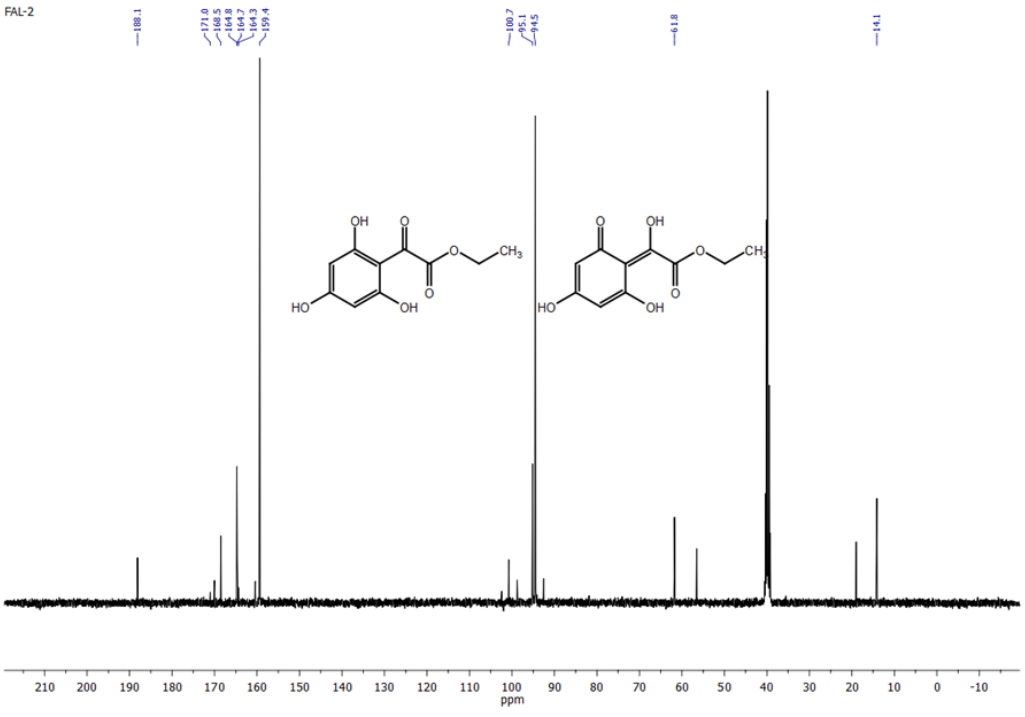
3.7 Ethyl 2,4,6-trihydroxyphenylglyoxylate (4)



This compound was prepared from 1 mmol of **1** with 1 mL EtOH according to preparation procedure of 2. Dull yellow solid, M.p. 196-198 ^{o}C, yield 0,215 g, 95%. ^{1}H NMR (400 MHz, DMSO, δ, ppm): 11.59, 8.95 (s, 3x1H, 3xOH), 5.84, 5.66 (s, 2x1H, =CH), 4.26 (q, *J* = 7.1 Hz, 2H, aromatic form`s $OCH_2$), 3.44 (q, *J* = 7.0 *Hz*, 2H, non-aromatic form`s OCH_2), 1.26 (t, *J* = 7.1 *Hz*, 3H, aromatic form`s $CH_3$), 1.05 (t, *J* = 7.0 *Hz*, 3H, non-aromatic form`s CH_3). ^{13}C NMR (101 MHz, DMSO, δ, ppm): 188.12 (Ar-C=O), 171.00, 170.00, 168.51, 164.76, 164.66, 164.35, 160.41, 159.35, 102.42, 100.73, 98.78, 95.15, 94.52, 92.54 (C=C and COOEt), 61.75, 56.52 (tautomeric form's OCH_2), 18.98, 14.13 (tautomeric form's CH_3). FTIR (ATR, cm^{-1}): 3462, 3178 (OH), 1721, 1632 (C=O). HRMS (ESI): *m/z* $[M - H]^+$ calcd. for $C_{10}H_{11}O_6^+$: 227.0550; found: 227.0562.

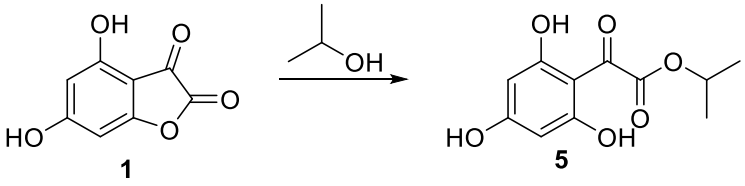


Scheme S7. ^{1}H NMR spectra of 4.

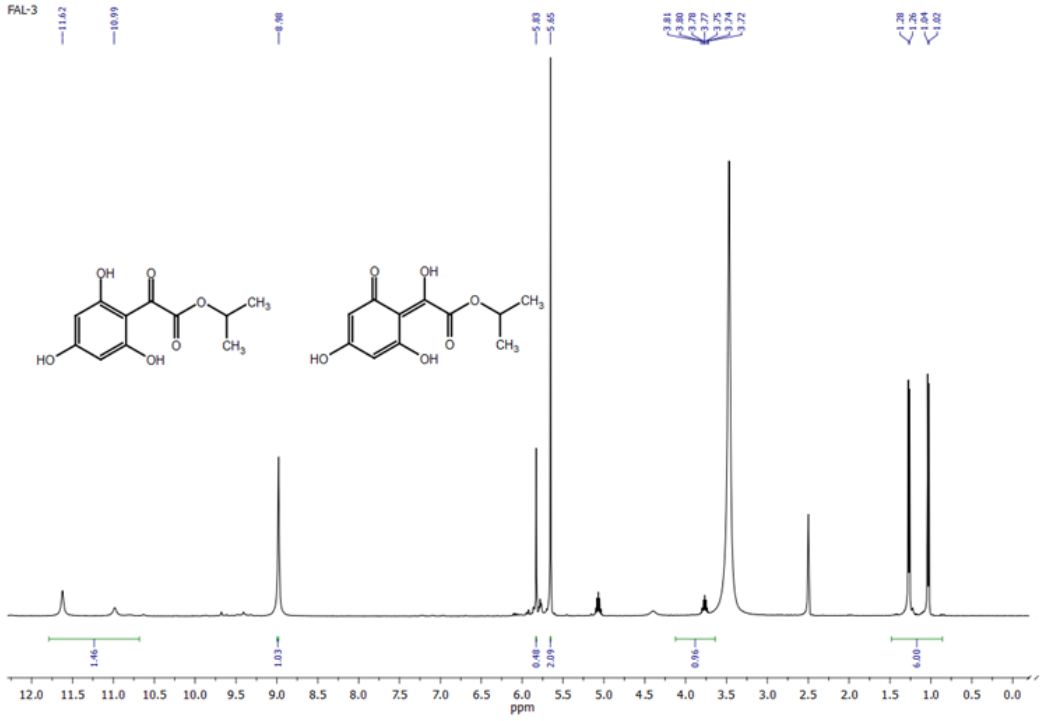


Scheme S8. ^{13}C NMR spectra of 4.

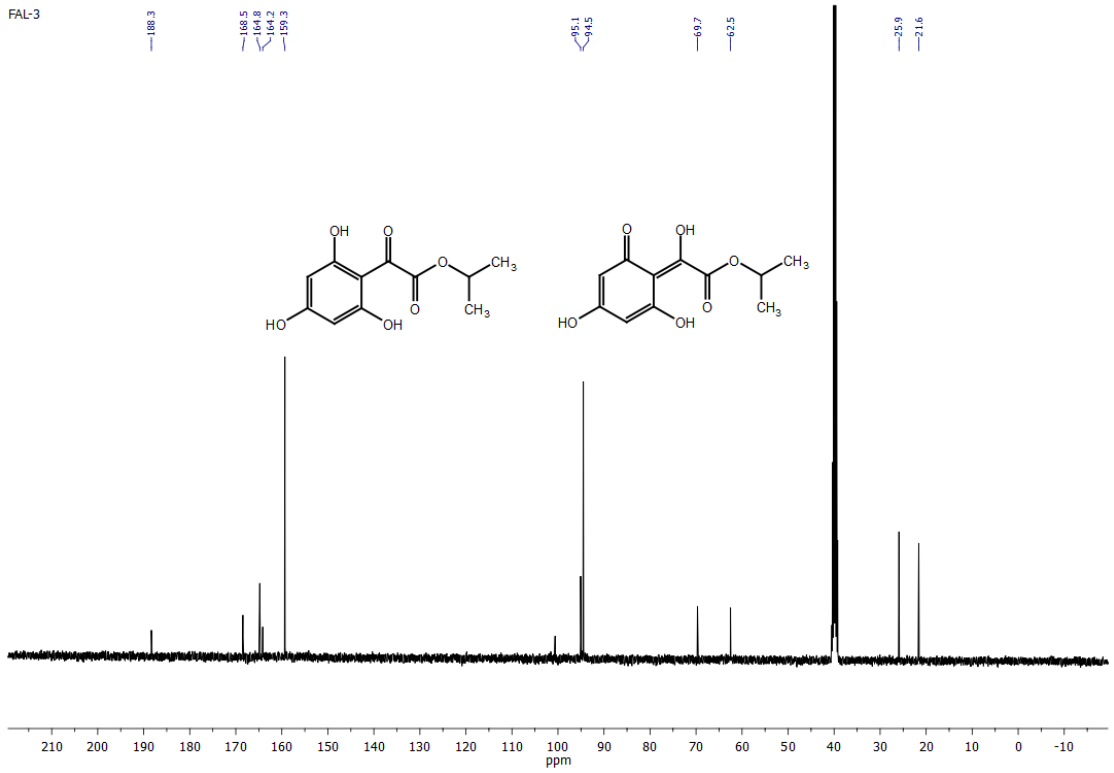
3.8 *i*-Propyl 2,4,6-trihydroxyphenylglyoxylate (5)



This compound was prepared from **1** mmol of 1 with 1 mL *i*-PrOH according to preparation procedure of **2**. Yellow solid, M.p. 213-215 ^{o}C, yield 0,212 g, 88%. ^{1}H NMR (400 MHz, DMSO, δ, ppm): 11.62, 10.98, 8.98 (s, 3H, 3xOH), 5.83, 5.65 (s, 2H, ArH), 5.07 (m, *J* = 6.4 Hz, 1H, $OCHMe_2$), 3.77 (m, *J* = 12.1, 6.0 Hz, 1H, $OCHMe_2$), 1.27 (d, *J* = 6.3 Hz, 6H, 2xCH_3), 1.03 (d, *J* = 6.1 Hz, 6H, 2xCH_3). ^{13}C NMR (101 MHz, DMSO, δ, ppm): 188.34 (Ar-C=O), 168.46, 164.79, 164.15, 159.34, 100.62, 95.08, 94.50 (C=C and COO*i*Pr), 69.69, 62.52 (tautomeric form's O<u>C</u>HMe_2), 25.90, 21.61(tautomeric form's CH_3). FTIR (ATR, cm^{-1}): 3465, 3171 (OH), 1719, 1633 (C=O)

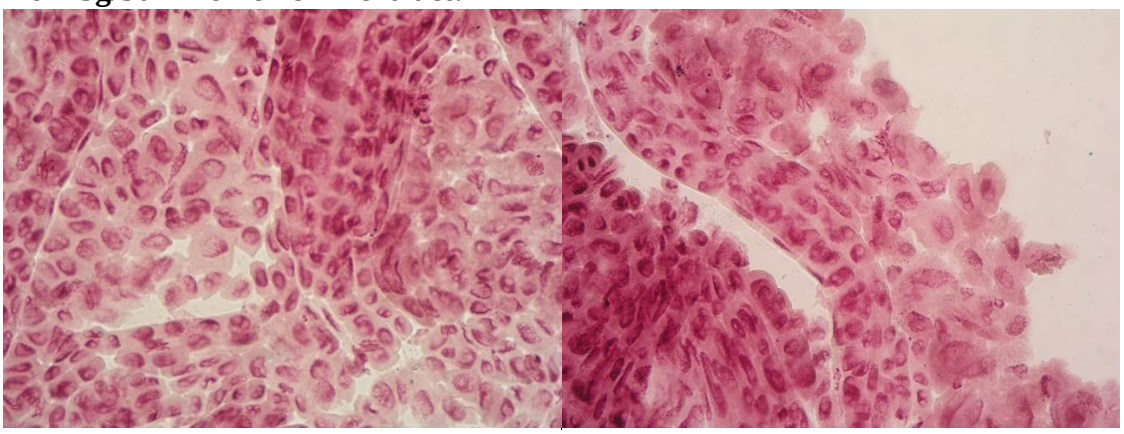


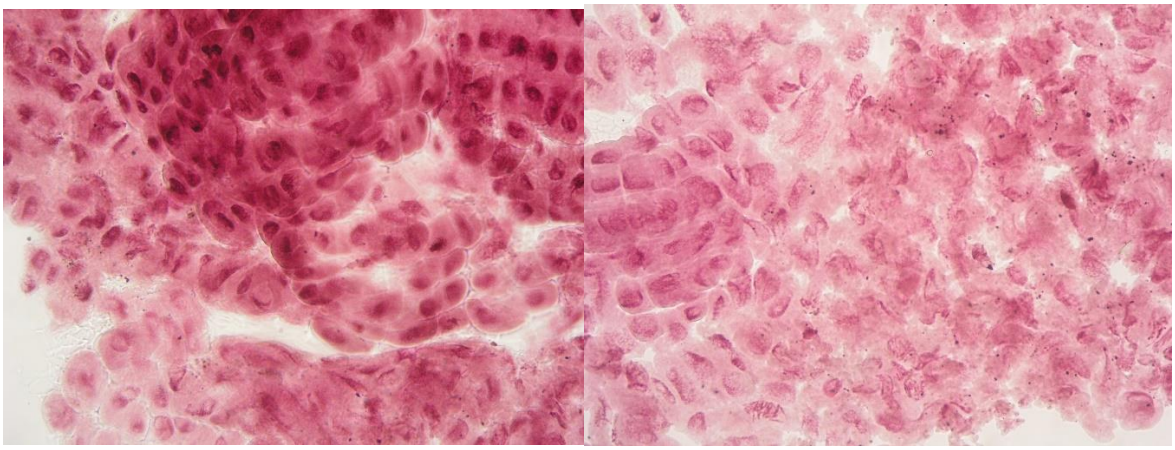
Scheme S9. ^{1}H NMR spectra of 5.

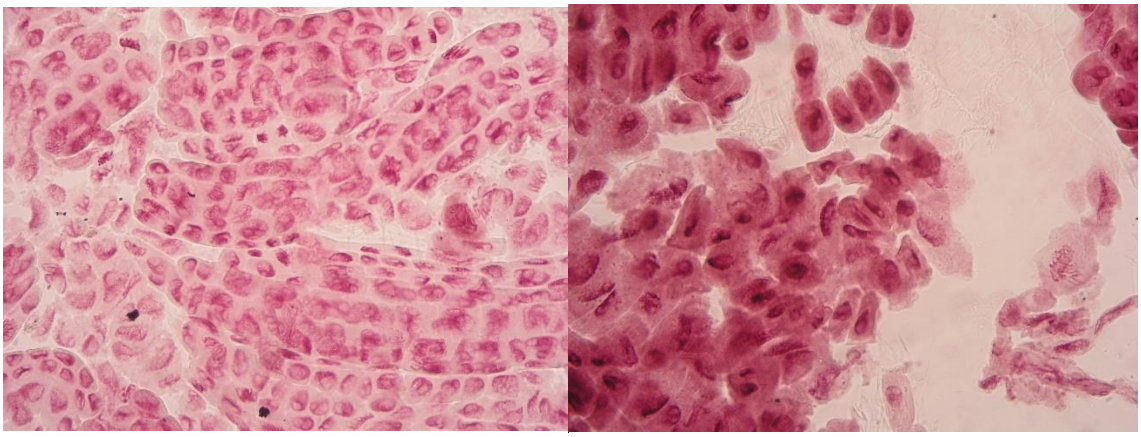


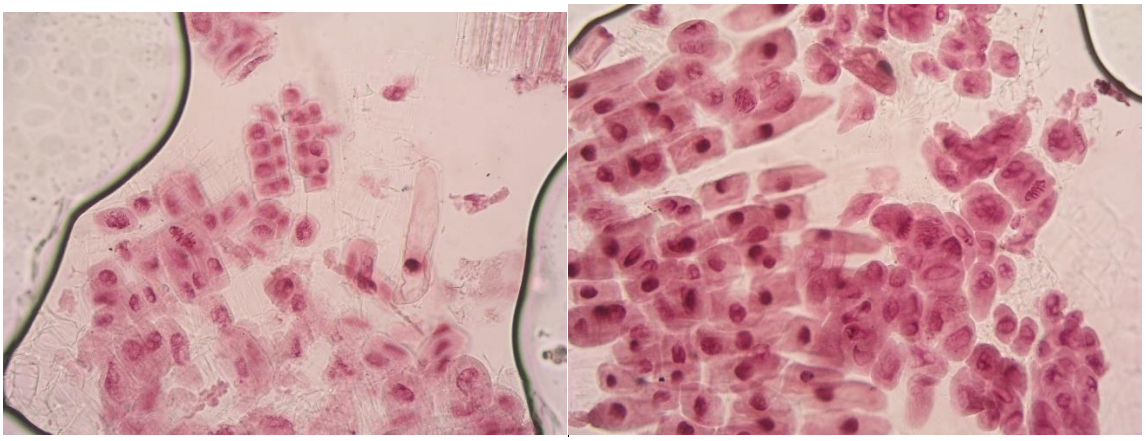
Scheme S10. ^{13}C NMR spectra of 5.

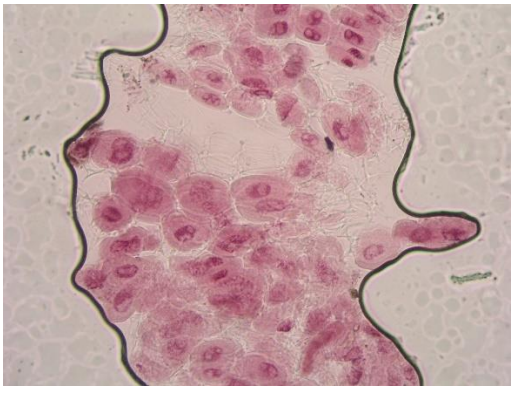
3.9 The meristematic cells images after exposure to 1 mL water extract prepared from 5g/50mL onion skin extract:



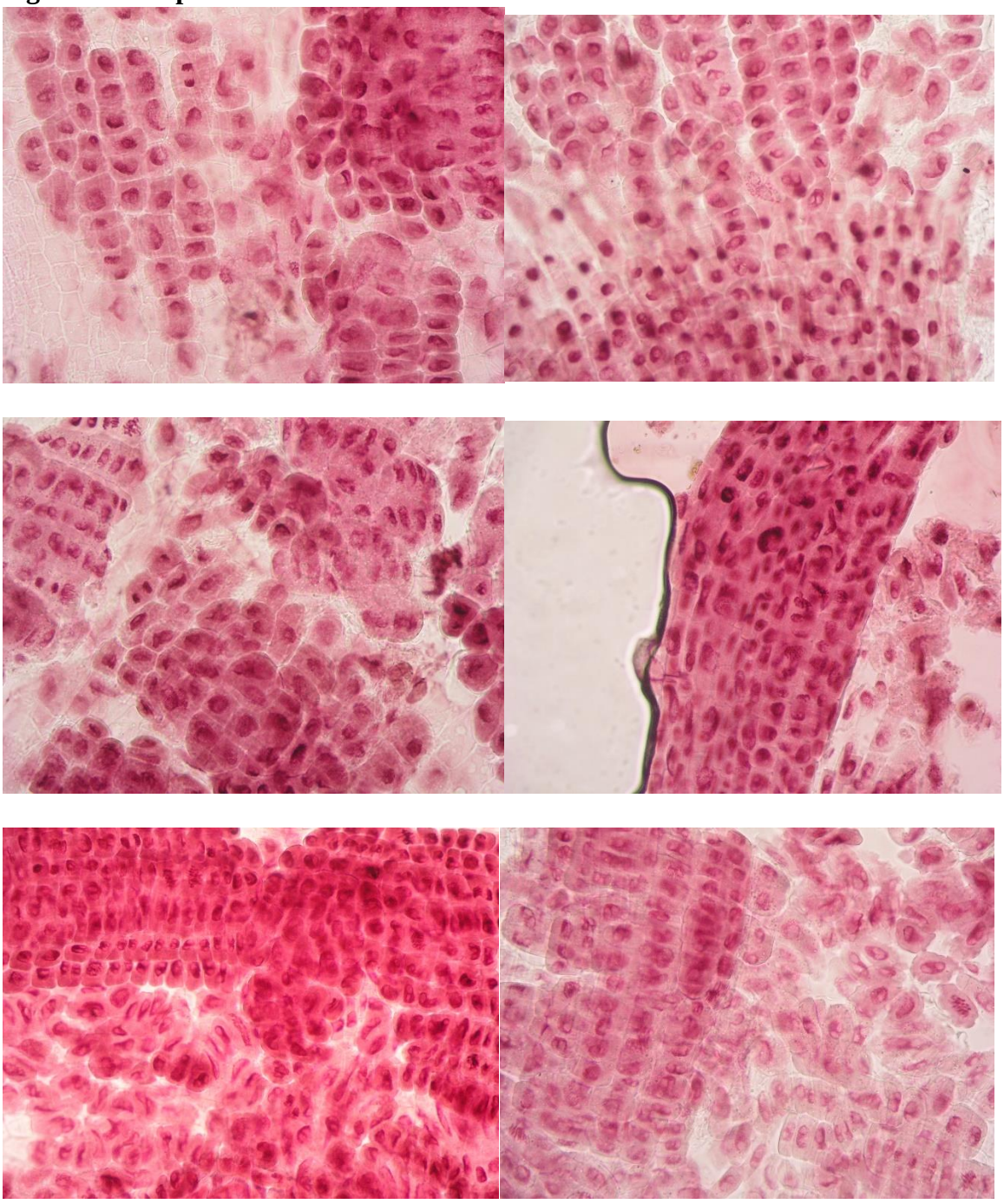


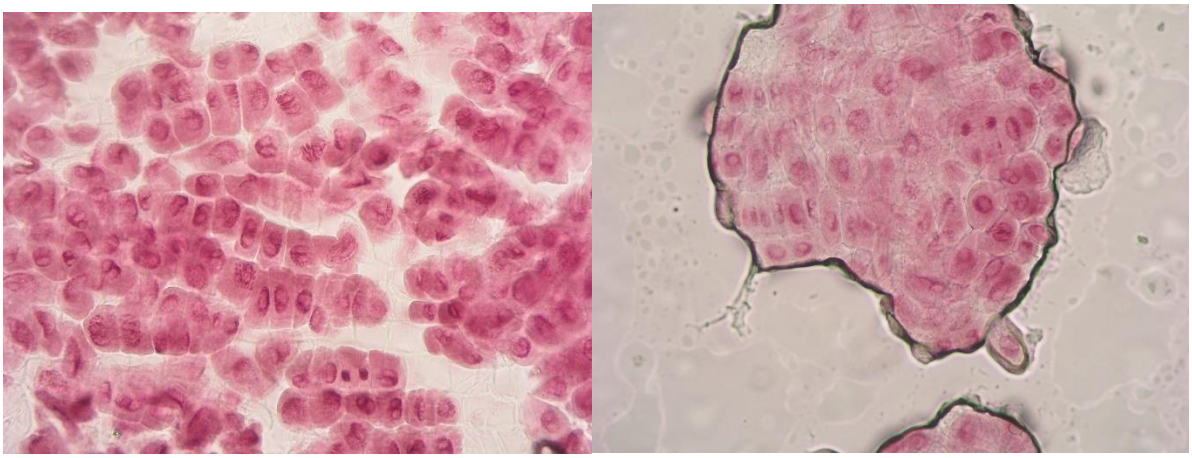




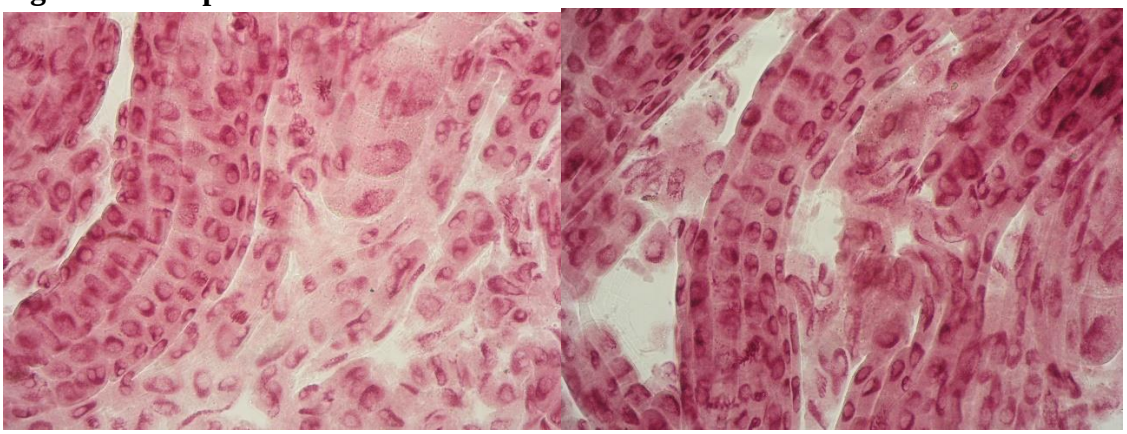


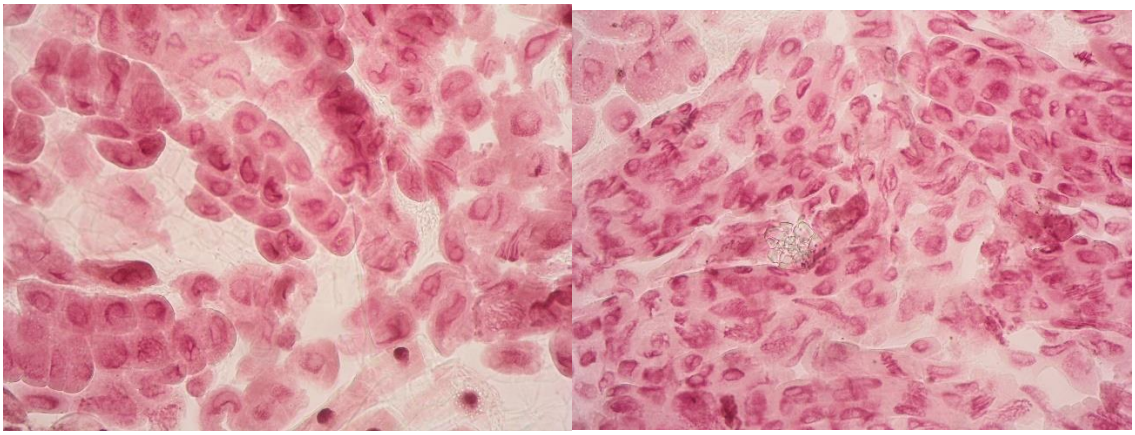
3.10 The meristematic cells images after exposure to 1 mL solution prepared from 1 mg/mL of compound 2:

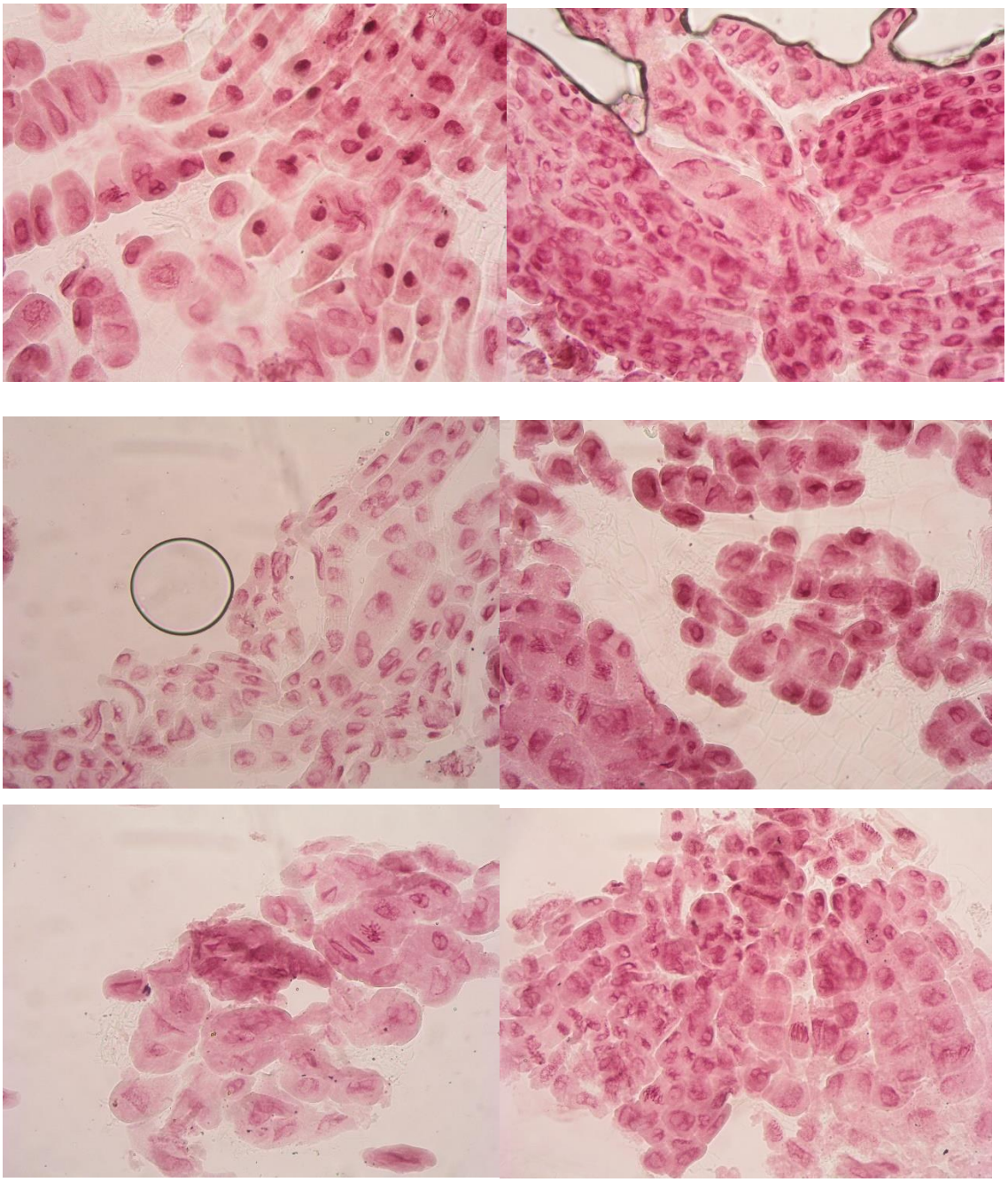




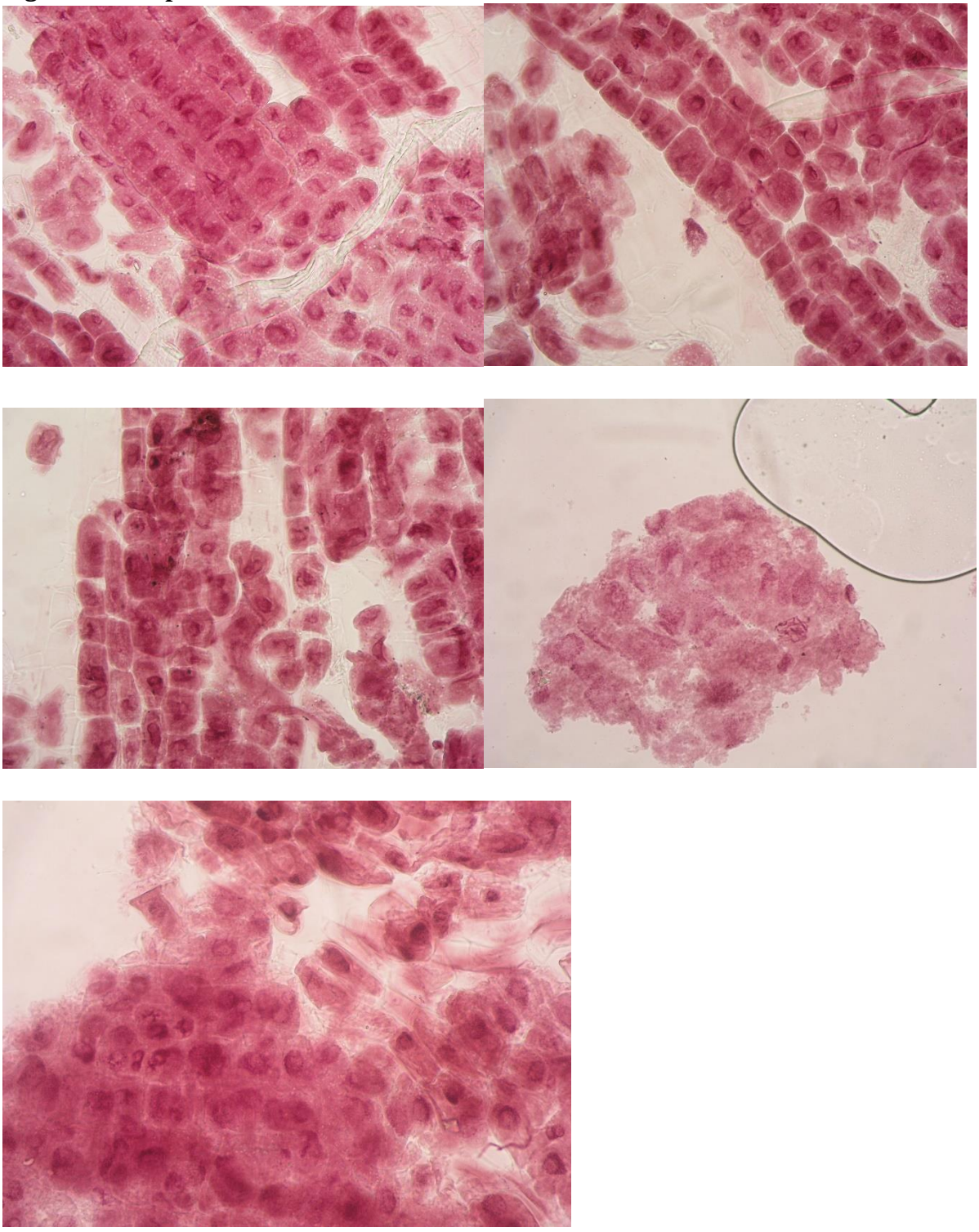
3.11 The meristematic cells images after exposure to 1 mL solution prepared from 2 mg/mL of compound 2:







3.12 The meristematic cells images after exposure to 1 mL solution prepared from 3 mg/mL of compound 2:



3.13 Control experiment; the meristematic cells images after exposure to 1 mL distilled water

