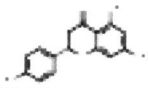
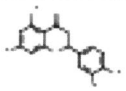
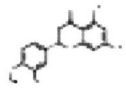
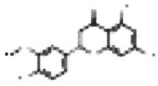
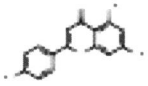
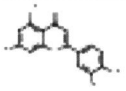
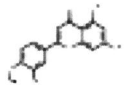
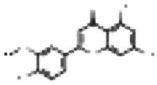
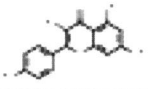
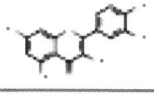
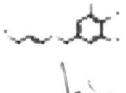
A 3, 4, 6, 7
B 4, 16, 17
C 8, 11

D 6, 7, 10, 17
E 3, 5, 7, 8, 12, 15, 16, 17
F 4, 15, 16

G 11, 13, 14
H 6, 9, 12

#	structure	$MolName	type
1		Naringenin	common
2		Eriodictyol	common
3 E / A		hesperetin	common
4 A B (F)		homoeriodictyol	common
5 E /		apigenin	common
6 H, D, A		luteolin	systematic
7 (15.2) (15.37) E, D, A		diosmetin	common
8 (15.4) (20) E, C		chrysoeriol	common
9 ~~A~~ H		para-coumaric acid	systematic
10 D		m-coumaric acid	systematic
11 C, G		o-coumaric acid	systematic
12 ~~H~~		caffeic acid	systematic
13 G		ferulic acid	common
14 E, G		iso-ferulic acid	systematic
15 F, E		kaempferol	common
16 ~~A~~ E, B, F		quercetin	common
17 B, E, D		myricetin	common

low
A B

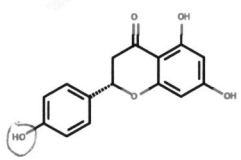
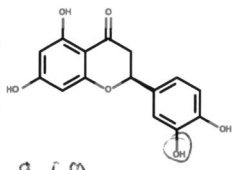
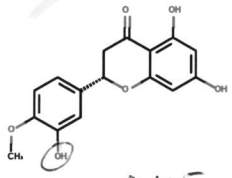
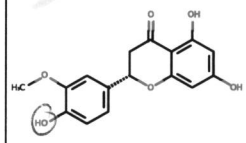
hig
E F + Mg

C D

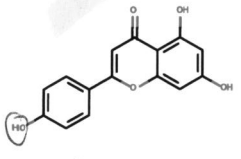
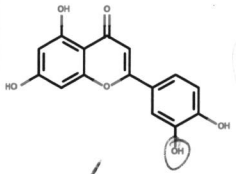
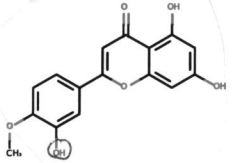
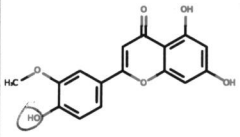
G H X - Mg

group

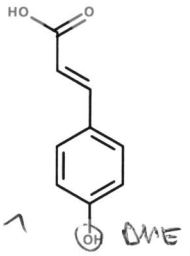
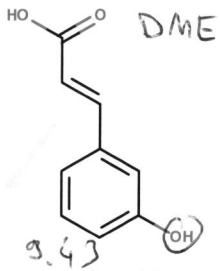
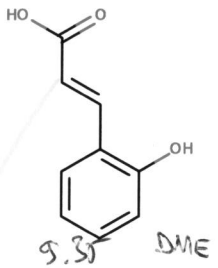
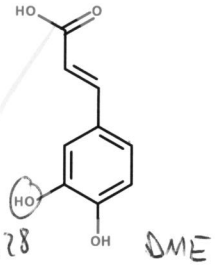
A

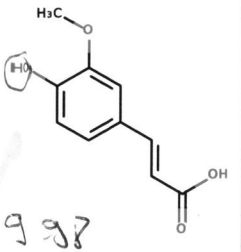
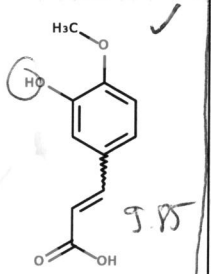
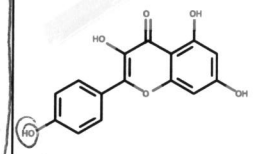
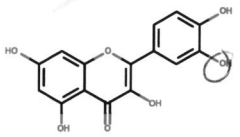
1	2	3	4
			
9.75 DME	9.69 DME	9.98 DME	10.06 DME
Naringenin	Eriodictyol	hesperetin	homoeriodictyol

B

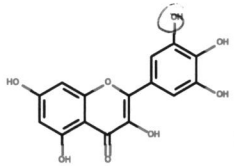
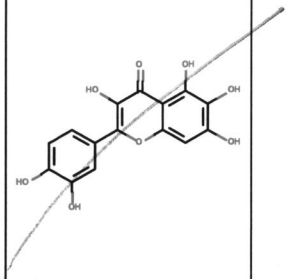
5	6	7	8
			
8.83 DME	✓ 12.16	9.57 ✓	9.24 ✓
apigenin	luteolin	diosmetin	chrysoeriol

C

9	10	11	12
			
9.57 DME	DME 9.43	9.35 DME	9.28 DME
para-coumaric acid	m-coumaric acid	o-coumaric acid	caffeic acid

13	14	15	16
			
9.98	✓ 9.85	8.63 DME	12.82 DME
ferulic acid	iso-ferulic acid	kaempferol	quercetin

D

17	18		
			
11.39 DME			
myricetin	quercetagetin		

Diarylheptanoid

Pinocembrin (7-methyl ether)

Lut. 100 mg final D106.5.7.2

Rotichrom TLC

$(\bar{y} - y_i)^2$

1 Introduction

Determination of the pH optimum of PFOMT with different substrates (caffeic acid, eriodictyol and iso-ferulic acid) and in low and high magnesium conditions. Measure progresscurve using six (6) timepoints (0, 3, 6, 9, 30 min and 2 h). Two different Mg^{2+}-concentrations (0 and 10 mM), as well as five pH-points (5.5, 6.5, 7.5, 8.5, 9.5). For each substrate this amounts to $6 \times 5 \times 2 = 60$ measurements.

2 Methods

2.1 Assays

The standard reaction conditions for a total volume of 50 μl are 50 mM MMT-buffer, 0.4 mM caffeic acid, 2.5 μM GSH, 0.5 mM SAM, $\pm$ **10 mM $MgCl_2$** and 20 μg PFOMT, plus 0.1 mM flavon as ITSD.

OMT-Reaction (50 μl):

volume	compound	final concentration
25 μl	100 mM buffer	50 mM
2 μl	10 mM caffeic acid	0.4 mM
1.25 μl	0.1 M GSH	2.5 μM
6.13 μl	81% 5 mM SAM (4.05 mM)	0.5 mM
10 μl	1 mg/ml PFOMT	0.2 μg/ μl
ad to 50 μl H_2O (5.62 μl)		

+0.5 µl with flavon

A standard substrate mastermix was prepared first. This mastermix was then added to an appropriate amount of buffer. 40 μl of this resulting *reaction buffer* was pipetted into an 1.5 mL centrifuge tube and the reaction was started by addition of PFOMT.

substrate mastermix A (40 $\times$ 15 μl):

volume	compound	final concentration
80 μl	10 mM caffeic acid	1.33 mM
50 μl	0.1 M GSH	8.33 μM
245.25 μl	81% 5 mM SAM	1.655 mM
20 μl	10 mM flavone	0.33 mM
ad to 600 μl H_2O (204.75 μl)		

substrate mastermix B (40 $\times$ 15 μl):

volume	compound	final concentration
80 μl	10 mM caffeic acid	1.33 mM
50 μl	0.1 M GSH	8.33 μM
245.25 μl	81% 5 mM SAM	1.655 mM
20 μl	1 M $MgCl_2$	33.3 mM
20 μl	10 mM flavone	0.33 mM
ad to 600 μl H_2O (184.75 μl)		

pH 5x

①	②	③	④	⑤
5.4	6.5	7.5	8.5	9.5

Mg^{++} 2x

Substrate 2x (3x)
Caffeic acid
ED
(iso-ferulic acid)

2.63 ml +
1.209 6

~~+860~~

4 ml

time points 6

~~0~~ ~~1~~ ~~5~~ ~~10~~ ~~45~~ ~~120~~

0 3 6 9 ~~12~~ ~~15~~ 120 (180)
30 2h

50µl 80x

~~+70~~
25µl buffer + 70x
10µl substrate mix

8 x 25

20µl

substrate mastermix C (40 × 15 µl):

volume	compound	final concentration
80 µl	10 mM eriodictyol	1.33 mM
50 µl	0.1 M GSH	8.33 µM
245.25 µl	81% 5 mM SAM	1.655 mM
20 µl	10 mM flavone	0.33 mM
ad to 600 µl H_2O (204.75 µl)		

substrate mastermix D (40 × 15 µl):

volume	compound	final concentration
80 µl	10 mM eriodictyol	1.33 mM
50 µl	0.1 M GSH	8.33 µM
245.25 µl	81% 5 mM SAM	1.655 mM
20 µl	1 M $MgCl_2$	33.3 mM
20 µl	10 mM flavone	0.33 mM
ad to 600 µl H_2O (184.75 µl)		

***reaction buffer* preparation:**

1. add 105 µl (7 × 15) *substrate-MM* to 175 µl (7 × 25) of 100mM of each *100 mM buffer (pH 5.4, 6.5, 7.5, 8.5, 9.5)*

Reaction:

1. 40 µl of *reaction buffer (2.1)* is pipetted into a 1.5 mL centrifuge tube, set at room temperature
2. reaction is started by addition of 10 µl 1 mg/mL PFOMT
3. incubate at 30 °C, 100 rpm
4. stop reaction by addition of 15 µl *stop solution* (10% TCA in 50% ACN)

Table 1: Sample times for activity test.

sample time t_i (min)	**addition of PFOMT** ($t_0 + \ldots$ min)	**stop time** (min)
$t_0 = 0$		
3		
6		
9		
30		
120		

	Start	3	6	9	~~Bo~~30	120
A1	0	3	6	9	30	120
A2	30	330	630	9.3	30.3	120.3
A3	1	4	7	10	31	121
A4	130	430	730	10.3	31.3	121.3
A5	2	5	8	11	32	122
~~A6~~	~~2.30~~					

bei 30°C statt Ei! → 10 min dann Ei!

B1	12	15	18	21	32	132
B2	12.3	15.3	18.3	21.3	32.3	132.3
B3	13	16	19	22	33	133
B4	13.3	16.3	19.3	22.3	33.3	133.3
B5	14	17	20	23	34	134

→ nach 18h 50 min

BC → nach 19h 50 min

20 µl Substrate mix

75 µl buffer (100 mM)
+ 15 µl Substrate mix
(0.3 mM flavon
1.3 mM substrate
8 µM GST
1.6 mM SAM

pH	f(pH 9)	ml pH 9
3.97	0	
4.22	0.25	0.5
4.56	0.41	0.82
5.13	0.58	1.16
6	0.75	1.5
7.19	0.92	1.84
10.09	1 (2 ml)	

Eppis @ 30°C / Substrate @ RT