

Contents

Abbreviations	5
Summary.....	9
Zusammenfassung.....	11
1 Introduction.....	13
1.1 Initial response: MAMP-triggered immunity.....	13
1.2 Secondary response: Effector-triggered immunity	14
1.3 Global response: systemic acquired resistance	15
1.4 The EDS1/PAD4 regulatory node.....	16
1.5 Flavin-dependent mono-oxygenase 1	18
1.5.1 FMO1 in EDS1/PAD4 dependent defence	19
1.5.2 FMO1 in systemic acquired resistance	20
1.5.3 FMO1 and cell death	21
1.5.4 FMO1 function.....	22
1.6 Chemical biology and screening strategies	23
1.7 Aims of this thesis.....	25
2 Screening of natural and synthetic compound libraries uncovers several putative activators ..	27
2.1 Screen for inhibitors	30
2.2 Screen for activators.....	31
2.2.1 Verification of activator chemicals	32
2.2.2 Confirmation of <i>FMO1</i> induction in wild-type seedlings by qRT-PCR	34
2.3 Thaxtomin A is a potent <i>FMO1</i> upregulator.....	37
2.3.1 Timeline and dose-dependence of thaxtomin A-induced <i>FMO1</i>	37
2.3.2 Thaxtomin A affects seedling growth, but not germination	39
2.3.3 Thaxtomin A does not trigger MAPK and ROS signalling	40
2.3.4 Analysis of perturbations in amino acid levels	42
2.3.5 Thaxtomin A leads to an increase in salicylic acid levels.....	44
2.3.6 Structure-activity relationship of thaxtomin A.....	44
2.3.7 Role of thaxtomin A in other signalling pathways.....	46
2.3.8 <i>FMO1</i> expression due to thaxtomin A is dependent on <i>EDS1</i> and <i>PAD4</i>	48
2.3.9 Thaxtomin A is a <i>PAD4</i> transcription activator.....	49
2.4 Monensin sodium salt and merbromin increase <i>FMO1</i> expression.....	53
2.4.1 <i>EC</i> ₅₀ determination	53
2.4.2 Monensin sodium salt and merbromin affect germination and growth in a concentration-dependent manner.....	54

2.4.3 Interaction between early defence signalling and <i>FMO1</i> upregulation	56
2.4.4 Role of monensin sodium salt in other signalling pathways.....	58
2.4.5 Analysis of changes in amino acid concentration	59
2.4.6 Effect of monensin sodium salt and merbromin on defence gene expression	60
2.4.7 Monensin sodium salt, but not merbromin leads to an increase in salicylic acid	62
2.4.8 Role of monensin sodium salt and merbromin in other signalling pathways	63
3 Discussion.....	65
3.1 Primary screening and hit selection.....	65
3.2 Origin and activity of thaxtomin A: a potent <i>FMO1</i> inducer	68
3.2.1 Structure-activity relationship of thaxtomin A bioactivity.....	70
3.2.2 Auxin inhibits thaxtomin A-induced <i>FMO1</i>	72
3.2.3 <i>FMO1</i> expression in plants: at death's door?	73
3.2.4 Positioning thaxtomin A activity within the signalling network	74
3.3 Merbromin and monensin sodium salt induce <i>FMO1</i> expression.....	76
3.4 Concluding Remarks.....	80
5 Materials & methods	83
5.1 Materials	83
5.1.1 Plant materials	83
5.1.2 Chemicals	84
5.1.3 Media	84
5.1.4 Buffers and solutions	84
5.2 Methods.....	85
5.2.1 Maintenance and cultivation of Arabidopsis plants	85
5.2.2 Chemical treatment of Arabidopsis plants	85
5.2.3 Confocal laser scanning microscopy (CLSM).....	85
5.2.4 Biochemical methods.....	86
5.2.5 Molecular biological methods.....	87
5.2.6 Reporter assays.....	90
5.2.7 Chemical screen	90
5.2.8 Monitoring the oxidative burst.....	91
5.2.9 Probe synthesis	91
References	93
Acknowledgements.....	105
Erklärung.....	107
Supplementary data.....	111