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Supporting Information for

Reinventing metabolic pathways: independent evolution of benzoxazinoids in flowering plants

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This PDF file includes:

SI Material and methods

Chemicals

All chemicals used in this study were purchased molecular biology grade or higher from Sigma Aldrich or Thermo Fisher unless otherwise stated.

Plant material and growth

Aphelandra squarrosa plants were grown in a greenhouse on a 14-h-light/ 10-h-dark photoperiod at 21 - 25°C during the day and 16 - 22°C during the night with 40 - 70 % humidity. *Lamium galeobdolon* plants were grown in a greenhouse on a 16-h-light/ 8-h-dark photoperiod at 20 - 24°C during the day and 16 - 20°C during the night with 45 - 60 % humidity. *Zea mays* cultivar "sweet nugget" plants used for BXD isolation were grown in a greenhouse on a 14-h-light/ 10-h-dark photoperiod at 21 - 24°C during the day and 19 - 22°C during the night with 40 - 70 % humidity. *Nicotiana benthamiana* plants used for transient gene expression were grown in a greenhouse on a 16-h-light/8-h-dark photoperiod at 22 °C and 60% relative humidity. Plants were grown for 3-4 weeks before infiltration of gene candidates.

Metabolite extraction and LC-MS metabolic analysis

Plant material was frozen in liquid nitrogen and ground to fine-powder in pre-chilled mortars or with 3 mm Tungsten Carbide Beads using a TissueLyser II (Quiagen). Tissue samples (100 mg ± 5%) were extracted with 500 µL methanol (LC-MS grade). Samples were vigorously vortexed and then incubated at 25°C with shaking for 15 min, followed by 15 min centrifugation at maximum speed using a table top centrifuge. Samples were then filtered with a 0.22 µm PTFE syringe filter before dilution for LC-MS analysis.

RNA-Seq and gene candidate identification

Total RNA from *A. squarrosa* and *Lamium sp.* tissues was extracted using RNeasy Mini Kit (Quiagen) according to manufacturer's instructions. RNase-Free DNase Set (Quiagen) was used to perform on-column genomic DNA digestion. RNA quality was assessed using a Nanophotometer N60 (Implen) as well as with a Bioanalyzer 2100 (BioRad). Three biological replicates of each tissue were prepared. Samples were submitted to Novogene for total RNA sequencing using the company's standard protocol for library preparation and Illumina RNA-sequencing. Approximately ≥ 40 million paired-end reads were acquired per sample. Reads were trimmed using the program OmicsBox (Biobam) and then assembled using the Trinity algorithm implemented in OmixBox and the CLC Genomics workbench (Qiagen)-integrated de novo assembly algorithm (bubble size, 800; minimum contig length, 500; mismatch cost, 2; insertion cost, 3; deletion cost, 3; length fraction, 0.8; similarity fraction, 0.9). The completeness of the generated *de novo* transcriptome assemblies was assessed by calculating the BUSCO value and the assembly used only if the value was ≥ 90%. The assembled transcriptomes were annotated using SwissProt 2021 database (blasting parameters: E-Value, 1.0E-3; number of Blast Hits, 20; word size, 3; low complexity filter, on; number of threads, 40; HSP length cutoff, 33). Reads were mapped on the annotated transcriptomes using CLC Genomics workbench (mapping parameters: mismatch cost, 2; insertion cost, 3; deletion cost, 3; length fraction, 0.8; similarity fraction 0.9; auto-detect paired distances, on; maximum number of hits for a read, 10). Gene candidate selection was performed by filtering the transcripts for enzymatic classes of interest and calculating the average RPKM expression value per tissue. Transcripts with a maximum RPKM value lower than 10 were removed. For the remaining transcripts, log fold change (logFC) between BXD-accumulating and non-accumulating tissues / species was calculated and the transcripts with LogFC < 1 were removed. Transcripts were then sorted from highest to lowest RPKM values.

Cloning

cDNA was prepared from total RNA with SuperScript IV VILO Master Mix (ThermoFisher Scientific) according to the manufacturer's instructions. Candidate genes were amplified from cDNA using Platinum SuperFi II PCR Master Mix (ThermoFisher Scientific). In the case of synthetic gene sequences obtained from Twist Biosciences, the synthesized sequences were used as template for PCR. PCR products were purified using DNA Clean and Concentrator-5 (Zymo). Amplified genes were inserted with In-Phusion HD

Cloning (Takara Bio) in the vectors p3Ω1 (*Bsa*I-HF digested), pOPINF (*Hind*III-HF/*Kpn*I-HF digested), or pESC-Leu (*Not*I-HF/*Pac*I-HF digested). *Escherichia coli* Top10 was transformed using the heat shock method and plated on LB agar-plates with appropriate selection. Overnight colonies were inoculated in liquid LB with appropriate selection and incubated at 37°C with 250 rpm. Plasmid DNA was isolated with Wizard Plus SV Minipreps DNA Purification System kit (Promega) and the inserted gene was fully sequenced. Primer sequences are given in SI Table 2.

A. tumefaciens*-mediated transient transformation of *Nicotiana benthamiana

Electrocompetent cells of *A. tumefaciens* GV3101 (Goldbio) were thawed on ice, mixed with 100 ng of sequence-confirmed plasmid, and incubated 30 min on ice. The cells were transferred into an electroporation cuvette (path, 1 cm) and electroporated using a Micropulser (BioRad). Transformed cells were recovered in 1 mL LB media and incubated for 3 h at 28°C, with 200 rpm shaking prior to plating on LB-agar plates with appropriate selection. Plates were incubated at 28°C for 48 h. Single colonies were inoculated in liquid LB with appropriate selection and incubated at 28°C, 200 rpm shaking over-night. For *N. benthamiana* transient-transformation, overnight cultures were pelleted by centrifugation at 4000 rpm for 10 min (14°C). The pellet was resuspended in infiltration media (10 mM MES, 10 mM $MgCl_2$, 100 µM acetosyringone, pH 5.7) to OD_{600}= 0.5-0.6 and incubated from 1.5 to 2.5 h at 28°C, 200 rpm. Isovolumes of the prepared infiltration solutions were mixed to obtain the desired co-infiltration mixtures. The co-infiltration mixtures were infiltrated in the abaxial side of 3-4 week old *N. benthamiana* leaves using a needless 1 mL syringe. Infiltrated plants were kept in the dark overnight and then transferred to a growth chamber and placed under growth lights. The infiltrated leaves were harvested 5 days post infiltration. In all transformations, a construct encoding the silencing repressor protein p19 was co-infiltrated to increase expression. When exogenous substrate was used, 1 mL of substrate dissolved in infiltration media (500 µM) was injected into transformed leaves three days after transformation with a needleless syringe.

Heterologous expression of candidate genes in *S. cerevisiae*

Saccharomyces cerevisiae WAT11 was made competent using the lithium acetate method (1). In brief, *S. cerevisiae* WAT11 was streaked to single colony on YPAD plates. A 10 mL YPAD seed culture inoculated with a single colony was grown overnight at 30°C, with shaking at 180 rpm. The seed culture was then topped with 40 mL YPAD and cultivated for 4-5 additional hours. After centrifugation (3000 x *g*, 4°C, 5 min), the pellet was washed with 25 mL ddH_2O and then again with 1 mL of ddH_2O. Cells were resuspended in 1 mL ddH_2O and 100 µL aliquots of the resuspended cells were transferred to a microcentrifuge tube and centrifuged at maximum speed for 1 min. The cell pellet was resuspended in the transformation mixture (240 µL PEG 3350 (50% w/v), 36 µL LiOAc (1 M), 100 µg salmon testis DNA, 1 µg plasmid DNA), incubated at 42°C for 50 min, centrifuged (6000 x *g*, room temperature, 1 min), resuspended again in 300 mL ddH_2O, and plated on SD-Leu agar plates containing 2% glucose. The plates were incubated at 30°C for 48-72 h. Single colonies were dissolved in 20 µL NaOH (20 mM) and boiled 10 min in a heat block. Two µL of the boiled colonies were added to a 15 µL PCR reaction with backbone-annealing primers to check for transformation.

Microsome preparation and microsome *in vitro* assay

Positive *S. cerevisiae* WAT11 colonies were inoculated in 30 mL SD-Leu medium (+ 2% Glucose) and incubated overnight at 30°C, 180 rpm. An aliquot of cell culture corresponding to OD_{600}=1 was added to 100 mL SD-Leu medium (+ 2% Glucose) and incubated for 30-35 h at 30°C, with shaking at 180 rpm. Cells were pelleted (5000 x *g*, 16°C, 5 min), the supernatant removed, and the pellet resuspended in 100 mL SD-Leu (+ 2% Galactose). Cultures were then incubated for 15-18 h at 30°C, with shaking at180 rpm. Cells were harvested by centrifugation (4000 x *g*, 4°C, 10 min), resuspended in 30 mL TEK buffer (50 mM Tris-HCl, 1 mM EDTA, 100 mM KCl, pH 7.5), then in 2 mL TES buffer (50 mM Tris-HCl, 1 mM EDTA, 600 mM sorbitol, 10g/l bovine serum albumin, 1.5 mM β-mercaptoethanol/100 mL, pH 7.5). Glass beads (500 µm diameter) were added and the cultures were manually shaken 5 x 1 min interspaced by 1 min cooling on ice. The beads were washed 3 times with 5 mL TES buffer and the supernatant fractions were pooled together and centrifuged (7500 x *g*, 4°C, 10 min). The supernatant was then centrifuged at 100,000 x *g*,

4°C, 90 min. The formed pellet was washed first with 2.5 mL TES buffer, then with 2.5 mL TEG buffer (50 mM Tris-HCl, 1 mM EDTA, 30% glycerol, pH 7.5), and ultimately with 1 mL TEG buffer. The solution was homogenized using a glass potter. Microsomes were stored at -20°C. An aliquot of microsomes (15 µL) were used for *in vitro* assays in KPO_4 buffer (25 mM, pH 7.5) with 1 mM substrate and 3 mM NADPH in 100 µL total volume. The reactions were incubated at 30°C, 300 rpm, for 2 h before quenching with an isovolume of methanol. Mixtures were incubated for 30 min on ice, centrifuged at maximum speed for 20 min, and filtered through a 0.22 µm PTFE syringe filter before LC-MS analysis.

Heterologous expression of candidate genes in *Escherichia coli* and *in vitro* assays

E. coli DE3 (ThermoFisher Scientific) cultures were transformed with sequence-confirmed plasmids using the heat-shock method, plated on LB-agar plates with appropriate selection and grown at 37°C overnight. Single colonies were inoculated in liquid LB medium with selection and grown at 37°C, 250 rpm, overnight. The seed culture (100 µL) was used to inoculate 100 mL 2 x YT medium with selection and the culture was grown at 37°C, 250 rpm shaking, until OD_{600} = 0.6-0.8. Cultures were then incubated at 18°C, 250 rpm, for 20 min before addition of 500 µM IPTG. Induced cultures were incubated at 18°C, 250 rpm, overnight. The cells were harvested by centrifugation (4000 x *g*, 4°C, 10 min), resuspended in 10 mL A1 buffer (50 mM TRIS-HCl, 50 mM glycine, 5% v/v glycerol, 0.5 M NaCl, 20 mM imidazole, pH 8) with 0.2 g / L lysozyme and 1 tablet / 50 mL buffer of EDTA-free protease inhibitor, and disrupted by sonication for 2 min (2 s on, 3 s off) on ice (Bandelin UW 2070). Cell debris was removed by centrifugation at 35,000 × *g* at 4°C for 20 min and the N-terminal His-tagged proteins were purified from the supernatant using NiNTA agarose (Qiagen) beads according to the manufacturer's instructions. Proteins were eluted using elution Buffer (A1 buffer + 500 mM imidazole, pH 8). Ultimately, elution buffer was exchanged for protein storage buffer (20 mM HEPES, 150 mM NaCl, pH 7.5) using Amicon 10 KDa concentrator columns (Merck Millipore). Proteins were stored at –20°C. In vitro assays were performed in KPO_4 buffer (25 mM, pH 7.5) containing 1 mM substrate, 1 µg protein, and variable cofactors depending on the enzyme tested. BX6 (2-ODD): 500 µM $FeSO_4$, 10 mM L-ascorbate, 10 mM 2-oxoglutarate, 3 mM DTT; BX7 (OMT): 1 mM SAM, 3 mM DTT; BX8 (UGT): 1.5 mM UDP-glucose, 100 µM $MgCl_2$.

Liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF) analysis

Samples were analyzed as described in (2, 3) with minor variations. Liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-qTOF) analysis were performed using a Thermo Scientific UltiMate 3000 ultra-high performance liquid chromatography (UHPLC) system coupled to an Impact II UHR-Q-ToF (Ultra-High Resolution QuadrupoleTime-of-Flight) mass spectrometer (Bruker Daltonics). Compounds were separated by reverse-phase liquid chromatography using a Phenomenex Kinetex XB-C18 column (100 x 2.1 mm, 2.6 µm; 100 Å) at 35 °C. The mobile phases for metabolite separation consisted of water with 0.1% formic acid (A) and acetonitrile (B). A flow rate of 0.3 mL/min was used for the chromatography with an injection volume of 2 µL. The chromatographic separation was performed starting at 5% B for 1 min, linear gradient from 5% to 50% B in 7 min, 100% B for 2.5 min, 5% B for 2.5 min. Authentic standards were prepared as 20 µM solutions in methanol and 2 µL aliquots were injected under the chromatographic conditions described above. Mass spectrometry acquisition was performed in positive or negative electrospray ionization mode depending on the compound of interest. For positive ionization mode, a capillary voltage of 3500 V and an end plate offset of 500 V were used; a nebulizer pressure of 2.8 bar was used, with nitrogen at 280 °C and a flow of 8 l/min as the drying gas. Acquisition was performed at 12 Hz in the mass range from *m/z* 100 to 1000, with data dependent MS2 and an active exclusion window of 1 min. For collision energy, the stepping option model (from 20 to 50 eV) was used. For negative ionization mode, a capillary voltage of 3500 V and an end plate offset of 500 V were used; a nebulizer pressure of 2.0 bar was used, with nitrogen at 250 °C and a flow of 10 l/min as the drying gas. Acquisition was performed at 12 Hz in the mass range from *m/z* 80 to 1000, with data dependent MS2 and an active exclusion window of 1 min. In both modes, for collision energy, the stepping option model (from 20 to 50 eV) was used. At the beginning of each sample run, a sodium formate-isopropanol calibration solution was directly injected in the source at 0.18 mL/hour using a syringe pump to calibrate MS spectra recorded. To avoid injection peak

and salt contamination of the MS, the initial 1 min of the active chromatographic gradient of each run was discarded to waste.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

Targeted analysis of BXDs was performed as previously described by (4) using an Agilent 1260 Infinity II LC system (Agilent Tecnologies) equipped with a ZORBAX Eclipse XDB-C18 column (50 x 4.6 mm, 1.8 µm; Agilent Technologies) coupled with a Sciex QTRAP 6500+ tandem mass spectrometer. Chromatographic conditions consisted of water with 0.05% formic acid (A) and acetonitrile (B) at a flow rate of 1.1 mL / min with a column temperature of 20°C and injection volume 2 µL. Chromatographic conditions consisted of: 0 – 0.5 min, 5% B; 0.5 – 6 min, 5 – 32.5% B; 6.02 – 7.0 min, 100% B; 7.10 – 9.5 min, 5% B. The mass spectrometer, equipped with a Turbospray ESI source, was operated in negative ionization mode. Multiple reaction monitoring was used to monitor analyte precursor ion – product ion transitions.

Synthesis of 3HI2O and HBOA

3HI2O was synthesized according to the method described in (5). In brief, sodium borohydride (385 mg, 10.2 mmol) was added in small portions to a stirred suspension of isatin (1 g, 6.8 mmol) in 40 mL of a 1:1 dichloromethane/ethanol mixture at 0 °C. The mixture was stirred at 0 °C until the suspension became colorless (about 30 min). Then water (20 mL) was added and the reaction mixture was stirred until bubbling stopped. The mixture was extracted with dichloromethane (3 x 20 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent evaporated under reduced pressure to yield 3HI2O (410 mg, 41%). HBOA was synthesized according to the method reported in (6). A solution of DIBALH in toluene (1 M, 2.46 mL, 2.46 mmol) was added within 5 min to a solution of 2,3-dioxo-1,4-benzoxazine (7) (200 mg, 1.23 mmol) in anhydrous toluene (5 mL) at –78 °C. The mixture was stirred for 2 h allowing it slowly to warm to room temperature. Then, the reaction was hydrolyzed by water (4 mL) at 0 °C. The mixture was acidified by 12 N HCl to pH 1. The aqueous layer was extracted with EtOAc (3 x 4 mL). The extracts were combined, dried with Na_2SO_4, and the solvents removed *in vacuo* to afford HBOA (126 mg, 63%). NMR spectra for synthesized 3HI2O and HBOA were measured on a 400 MHz Bruker Avance III HD spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). CD_3OD and $CDCl_3$ were used as solvents. NMR spectra were referenced to the residual solvent signals at δ_H 3.31 and δ_C 49.0 for CD_3OD and δ_H 7.26 and δ_C 77.0 for $CDCl_3$. For spectrometer control and data processing, Bruker TopSpin ver. 3.6.1 was used. The spectroscopic data for 3HI2O and HBOA are in accordance with the values reported in the literature (5, 8). NMR data for 3HI2O and HBOA are given in SI data set 2.

Isolation of HBOA-Glc, DIBOA-Glc, TRIBOA-Glc, and DIMBOA-Glc

HBOA-Glc was enzymatically synthesized by incubating LgBX8 with synthetically prepared HBOA, using the same reaction conditions described above for the *in vitro* assay. Ninety small scale reactions were combined and concentrated on a rotavapor prior to purification using preparative HPLC. DIBOA-Glc was isolated from 25 g of *L. galeobdolon* leaves. Leaves frozen in liquid nitrogen were ground in pre-cooled mortars and then extracted with three volumes of methanol, incubated at 25°C for 15 min, and then centrifuged at 4000 x *g* for 10 min. The methanol fraction was dried in a rotavapor and then re-extracted with 60% methanol in water. This fraction was purified on a SPE column (Oasis PRIME HLB 6cc- 500mg-Extraction Cartridge, Waters). The SPE-purified fraction was diluted 1:2 with methanol (60%) prior to preparative HPLC injection. TRIBOA-Glc was obtained enzymatically by using AsBX6 as described for the in vitro enzymatic assays by combining 50 small scale reactions. Reactions were combined, concentrated on a rotavapor, and injected on a preparative HPLC. DIMBOA-Glc was isolated from *Zea mays* 'Sweet Nugget' two weeks-old seedlings as described for DIBOA-Glc isolation. Preparative-scale chromatographic separation was performed using an Agilent 1260 Infinity II preparative HPLC system coupled to a multiple wavelength detector and fraction collector. A Phenomenex Kinetex XB-C18 column (250 x 10 mm, 5 µm, 100 A) was used. The mobile phases used for separation were A (H_2O + 0.1% formic acid) and B (acetonitrile). The flow rate was set at 6.0 mL/min with a gradient of B from 5% to 21% in 21 min, 100% B for 5 min, 5% B for 5 min. Manual injections (300 µL per injection) were performed, and the separation was monitored at 254 nm and 280 nm simultaneously.

NMR analysis of enzymatic products and isolated compounds
NMR measurements of enzymatically produced compounds were carried out on a 700 MHz Bruker Avance III HD spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany), equipped with a TCI cryoprobe using standard pulse sequences as implemented in Bruker Topspin ver. 3.6.1. (Bruker Biospin GmbH, Rheinstetten, Germany). Chemical shifts were referenced to the residual solvent signals of methanol-d_3 (δ_H 3.31/δ_C 49.0) and acetone-d_6 (δ_H 2.05/δ_C 29.84), respectively. All NMR data are given in SI data set 2.

ECD measurement and data comparison
ECD spectra of HBOA-Glc, DIBOA-Glc, TRIBOA-Glc, and DIMBOA-Glc were measured at 25°C on a JASCO J-810 spectropolarimeter (JASCO cooperation, Tokyo, Japan) using a 350 µL cell. Instrument control and data processing was accomplished using JASCO Spectra Manager II. Experimentally measured ECD data and calculated data were compared using SpecDis ver.1.71 (https://specdis-software.jimdo.com) and indicates *R* configuration for all compounds tested (SI Fig. 10). In addition, *R* configuration of DIBOA-Glc was further proved by measuring the specific optical rotation on a Jasco P-1030 polarimeter.

Supplementary material and methods references

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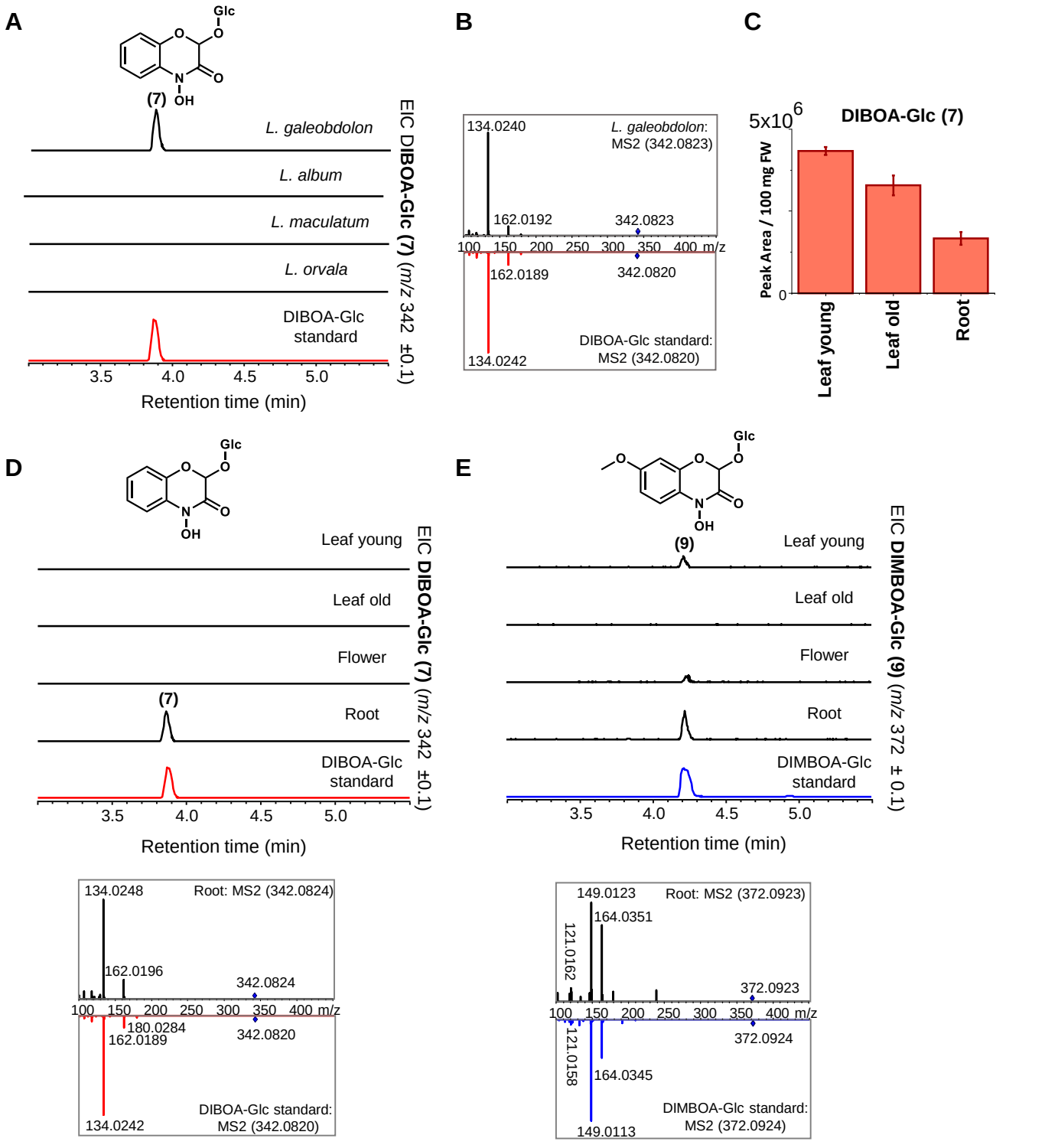


SI Figure 1: Metabolic profiling of *A. squarrosa* and *L. galeobdolon*. A) LC-qTOF analysis of *Lamium sp.* methanol leaf extracts. DIBOA-Glc presence was confirmed with an authentic standard. B) MS2 fragmentation spectra of DIBOA-Glc in *L. galeobdolon* matches the MS2 fragmentation spectra of the reference standard. C) Tissue distribution of DIBOA-Glc accumulation in *L. galeobdolon*. Means ± SE (n = 3 biological replicates) are shown. D and E) DIBOA-Glc and DIMBOA-Glc content in *A. squarrosa* organs. Plant material was extracted with methanol and analyzed using LC-qTOF. MS2 fragmentation spectra of DIBOA-Glc and DIMBOA-Glc matched those of the corresponding authentic standards.

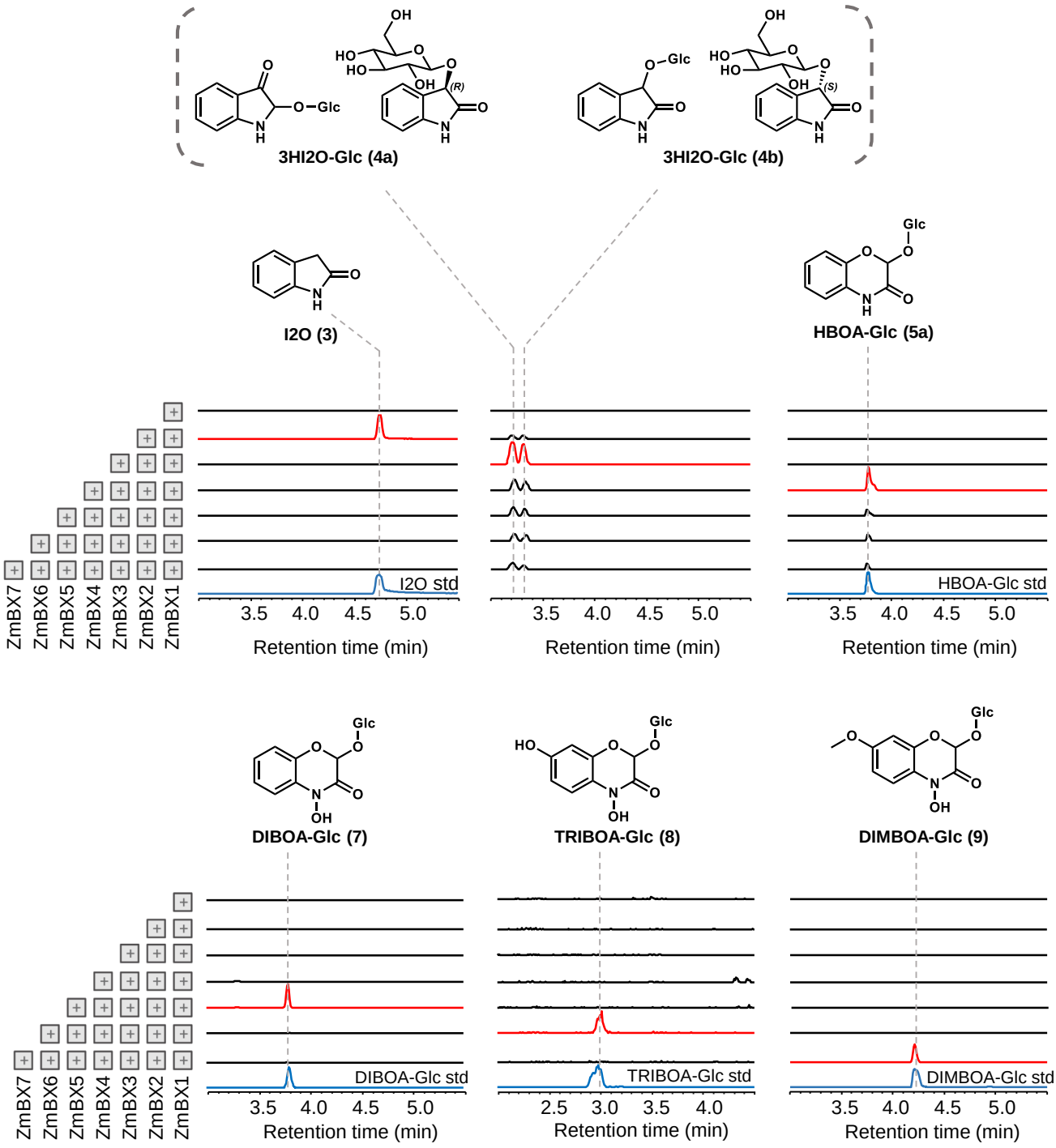
A

Root	Young leaf	Flower	Fully developed leaf	Gene	Annotation	LogFC
1.0	0.1	0.4	0.2	TRINITY_DN4480_c0_g1_i1	Cytochrome P450 CYP75	2.45
0.8	1.0	0.2	0.1	TRINITY_DN6556_c0_g1_i1	Flavin-containing monooxygenase	3.28
0.7	1.0	0.1	0.1	TRINITY_DN5839_c0_g1_i1	O-methyltransferase	3.91
0.1	1.0	0.8	0.0	TRINITY_DN13400_c0_g1_i1	2-oxoglutarate dependent dioxygenase	6.33
1.0	0.3	0.3	0.0	TRINITY_DN1230_c0_g1_i1	Polyphenol oxidase	6.53
0.4	0.1	1.0	0.3	TRINITY_DN5296_c0_g1_i1	Cytochrome P450 CYP94	1.81
1.0	0.1	0.2	0.0	TRINITY_DN2569_c0_g1_i1	Cytochrome P450 CYP81	21.18
1.0	0.0	0.0	0.0	TRINITY_DN12675_c0_g1_i1	Cytochrome P450 CYP71	9.30
0.4	1.0	0.5	0.4	TRINITY_DN5057_c0_g1_i2	Cytochrome P450 CYP98	1.31
1.0	0.0	0.0	0.0	TRINITY_DN5547_c0_g1_i1	Cytochrome P450 CYP71	7.66
1.0	0.4	0.2	0.1	TRINITY_DN15617_c0_g1_i1	Polyphenol oxidase	2.76
1.0	0.1	0.7	0.0	TRINITY_DN2569_c0_g1_i2	Cytochrome P450 CYP81	10.89
0.8	1.0	0.8	0.4	TRINITY_DN6106_c0_g1_i1	O-methyltransferase	1.45
1.0	0.0	0.0	0.0	TRINITY_DN1798_c0_g1_i1	O-methyltransferase	20.32
1.0	0.3	0.3	0.3	TRINITY_DN3039_c0_g1_i1	UDP-glucose glucosyltransferase	1.68
1.0	0.0	0.0	0.0	TRINITY_DN11257_c0_g1_i2	Cytochrome P450 CYP71	4.48
1.0	0.3	0.2	0.5	TRINITY_DN1237_c0_g1_i2	UDP-glucose glucosyltransferase	1.12
0.2	1.0	0.5	0.4	TRINITY_DN2282_c0_g1_i2	Cytochrome P450 CYP97	1.39
0.5	1.0	0.8	0.1	TRINITY_DN2711_c0_g1_i2	Cytochrome P450 CYP51	2.75
1.0	0.0	0.1	0.0	TRINITY_DN4762_c0_g1_i1	Polyphenol oxidase	7.61
1.0	0.6	0.0	0.2	TRINITY_DN11613_c0_g2_i1	Cytochrome P450 CYP78	2.69
1.0	0.1	0.2	0.3	TRINITY_DN2021_c0_g1_i4	Cytochrome P450 CYP72	1.62
1.0	0.2	0.5	0.0	TRINITY_DN13074_c0_g1_i1	Cytochrome P450 CYP72	6.15
1.0	0.6	0.5	0.5	TRINITY_DN2396_c1_g2_i2	UDP-glucose glucosyltransferase	1.02
1.0	0.0	0.0	0.0	TRINITY_DN4025_c0_g1_i2	Cytochrome P450 CYP71	7.91
1.0	0.0	0.5	0.1	TRINITY_DN9900_c0_g1_i2	Cytochrome P450 CYP749	3.04
1.0	0.0	0.0	0.4	TRINITY_DN2173_c0_g1_i3	Cytochrome P450 CYP76	1.25
1.0	0.3	0.2	0.0	TRINITY_DN238_c0_g1_i2	O-methyltransferase	7.58
1.0	0.0	0.0	0.0	TRINITY_DN121516_c0_g1_i1	Dioxygenase	10.98
1.0	0.4	0.6	0.0	TRINITY_DN13074_c0_g1_i2	O-methyltransferase	4.61
1.0	0.3	0.4	0.3	TRINITY_DN13693_c0_g1_i1	2-oxoglutarate dependent dioxygenase	1.56
1.0	0.5	0.5	0.5	TRINITY_DN1155_c0_g2_i3	Cytochrome P450 CYP72	1.12
1.0	0.0	0.0	0.0	TRINITY_DN12179_c0_g1_i3	Cytochrome P450 CYP76	7.13
1.0	0.3	0.2	0.4	TRINITY_DN1237_c0_g1_i1	UDP-glucose glucosyltransferase	1.41
1.0	0.0	0.0	0.0	TRINITY_DN9958_c0_g1_i1	O-methyltransferase	18.48
1.0	0.2	0.0	0.0	TRINITY_DN15617_c1_g1_i3	Polyphenol oxidase	18.47
1.0	0.0	0.1	0.0	TRINITY_DN7424_c0_g1_i3	Cytochrome P450 CYP71	18.45
1.0	0.0	0.0	0.0	TRINITY_DN14114_c0_g2_i3	2-oxoglutarate dependent dioxygenase	7.81
1.0	0.1	0.1	0.1	TRINITY_DN6380_c0_g1_i11	Cytochrome P450 CYP76	3.60
1.0	0.0	0.0	0.1	TRINITY_DN14457_c0_g1_i3	Cytochrome P450 CYP71	4.05
1.0	0.0	0.0	0.0	TRINITY_DN60557_c0_g1_i1	2-oxoglutarate dependent dioxygenase	9.37
1.0	0.3	0.0	0.0	TRINITY_DN10769_c0_g2_i1	Cytochrome P450 CYP87	8.43
1.0	0.0	0.0	0.0	TRINITY_DN10533_c0_g1_i3	2-oxoglutarate dependent dioxygenase	18.01
1.0	0.0	0.0	0.0	TRINITY_DN2404_c0_g2_i1	Cytochrome P450 CYP749	17.93
1.0	0.0	0.2	0.0	TRINITY_DN4762_c0_g1_i3	Polyphenol oxidase	6.33
1.0	0.1	0.0	0.0	TRINITY_DN9958_c0_g1_i4	O-methyltransferase	7.99
1.0	0.0	0.0	0.0	TRINITY_DN1798_c0_g2_i4	O-methyltransferase	5.99
1.0	0.0	0.0	0.0	TRINITY_DN238_c0_g2_i2	O-methyltransferase	17.88
1.0	0.0	0.6	0.0	TRINITY_DN13254_c0_g1_i1	Cytochrome P450 CYP76	5.63
1.0	0.2	0.2	0.2	TRINITY_DN801_c0_g1_i13	Cytochrome P450 CYP749	2.49
1.0	0.2	0.2	0.0	TRINITY_DN10074_c0_g1_i1	Cytochrome P450 CYP71	4.69
1.0	0.0	0.0	0.0	TRINITY_DN11835_c0_g1_i1	Cytochrome P450 CYP86	6.03
1.0	0.4	0.2	0.1	TRINITY_DN311_c0_g1_i4	2-oxoglutarate dependent dioxygenase	3.28
1.0	0.0	0.0	0.0	TRINITY_DN41106_c0_g1_i1	2-oxoglutarate dependent dioxygenase	17.76
1.0	0.3	0.2	0.4	TRINITY_DN4825_c0_g1_i2	Cytochrome P450 CYP71	1.32
1.0	0.2	0.2	0.3	TRINITY_DN801_c0_g1_i14	Cytochrome P450 CYP749	1.99
1.0	0.7	0.1	0.0	TRINITY_DN8686_c0_g1_i1	O-methyltransferase	10.08
1.0	0.1	0.0	0.0	TRINITY_DN5839_c1_g1_i1	O-methyltransferase	8.26
1.0	0.0	0.0	0.0	TRINITY_DN151_c1_g1_i1	Cytochrome P450 CYP716	17.65
1.0	0.4	0.1	0.2	TRINITY_DN21159_c0_g1_i1	O-methyltransferase	2.15
1.0	0.0	0.0	0.0	TRINITY_DN19747_c0_g1_i1	2-oxoglutarate dependent dioxygenase	7.73
1.0	0.1	0.0	0.0	TRINITY_DN5839_c0_g2_i1	O-methyltransferase	17.54
1.0	0.3	0.4	0.4	TRINITY_DN9349_c0_g1_i1	UDP-glucose glucosyltransferase	1.36
1.0	0.0	0.0	0.0	TRINITY_DN13750_c0_g1_i1	2-oxoglutarate dependent dioxygenase	17.50
1.0	1.0	0.1	0.1	TRINITY_DN5839_c0_g1_i2	O-methyltransferase	3.72
1.0	0.6	0.0	0.1	TRINITY_DN11613_c0_g3_i1	Cytochrome P450 CYP78	3.08
1.0	0.0	0.0	0.0	TRINITY_DN9958_c0_g1_i2	O-methyltransferase	9.15
1.0	0.1	0.0	0.0	TRINITY_DN238_c0_g2_i1	O-methyltransferase	6.59
1.0	0.4	0.0	0.2	TRINITY_DN14053_c0_g1_i1	Cytochrome P450 CYP80	2.43
1.0	0.0	0.0	0.0	TRINITY_DN4282_c0_g2_i1	Hydroxylase	17.34
1.0	0.0	0.2	0.0	TRINITY_DN10530_c0_g1_i1	Cytochrome P450 CYP86	5.65
1.0	0.0	0.1	0.1	TRINITY_DN13419_c0_g3_i1	UDP-glucose glucosyltransferase	2.90
1.0	0.0	0.0	0.0	TRINITY_DN908_c0_g1_i8	Dioxygenase	17.29
1.0	0.0	0.0	0.0	TRINITY_DN2404_c0_g1_i4	Cytochrome P450 CYP749	17.16
1.0	0.0	0.0	0.0	TRINITY_DN908_c0_g1_i3	Dioxygenase	7.21
1.0	0.0	0.2	0.3	TRINITY_DN8603_c0_g1_i2	Cytochrome P450 CYP83	1.77
1.0	0.6	0.7	0.1	TRINITY_DN51606_c0_g1_i1	O-methyltransferase	2.83
1.0	0.3	0.3	0.3	TRINITY_DN801_c0_g1_i10	Cytochrome P450 CYP749	1.98
1.0	0.1	0.5	0.0	TRINITY_DN12172_c0_g1_i1	O-methyltransferase	4.71
1.0	0.0	0.0	0.0	TRINITY_DN8982_c0_g1_i2	O-methyltransferase	16.96
1.0	0.0	0.1	0.0	TRINITY_DN6380_c0_g1_i1	Cytochrome P450 CYP76	4.79
1.0	0.1	0.0	0.0	TRINITY_DN908_c0_g1_i17	Dioxygenase	6.27
1.0	0.0	0.1	0.0	TRINITY_DN6380_c0_g1_i10	Cytochrome P450 CYP76	4.85
0.9	0.6	1.0	0.3	TRINITY_DN5058_c0_g1_i1	Indole-3-glycerol phosphate lyase	1.97
1.0	0.0	0.0	0.0	TRINITY_DN4025_c0_g1_i1	Cytochrome P450 CYP71	16.87
1.0	0.0	0.1	0.0	TRINITY_DN22668_c0_g1_i1	Cytochrome P450 CYP96	4.71
1.0	0.0	0.0	0.0	TRINITY_DN9958_c0_g1_i3	O-methyltransferase	16.76
1.0	0.0	0.1	0.0	TRINITY_DN134475_c0_g1_i1	O-methyltransferase	16.75
1.0	0.0	0.0	0.0	TRINITY_DN4282_c0_g1_i1	Hydroxylase	16.72
1.0	0.5	0.5	0.4	TRINITY_DN971_c0_g1_i3	Dioxygenase	1.34
1.0	0.0	0.0	0.1	TRINITY_DN14799_c0_g1_i4	2-oxoglutarate dependent dioxygenase	3.66

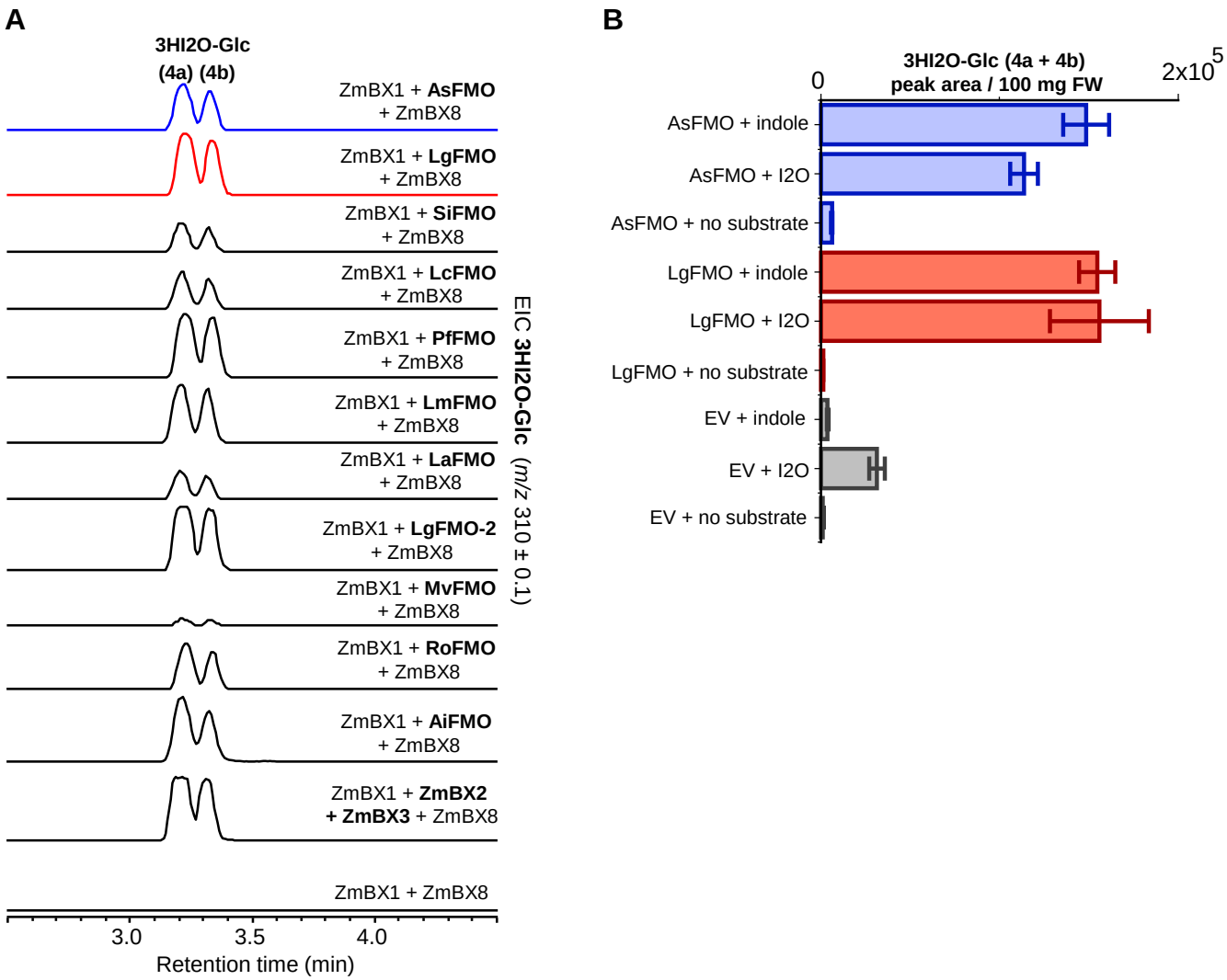
B

L. galeobdolon	*L. album*	*L. maculatum*	*L. orvala*	Gene	Annotation	LogFC
1.0	0.0	0.1	0.1	Contig_31519	Cytochrome P450 CYP72	3.95
1.0	0.1	0.1	0.2	Contig_13076	Cytochrome P450 CYP92	3.51
1.0	0.1	0.4	0.0	Contig_17476	Cytochrome P450 CYP72	2.70
1.0	0.0	0.0	0.0	Contig_31522	Cytochrome P450 CYP72	6.88
1.0	0.4	0.4	0.1	Contig_16829	Cytochrome P450 CYP72	1.67
1.0	0.0	0.0	0.0	Contig_8868	Cytochrome P450 CYP71	8.81
1.0	0.0	0.0	0.0	Contig_11095	Flavin-containing monooxygenase	7.79
1.0	0.0	0.0	0.0	Contig_30622	Cytochrome P450 CYP72	8.33
1.0	0.0	0.0	0.0	Contig_2002	Cytochrome P450 CYP72	8.31
1.0	0.0	0.0	0.0	Contig_14367	Cytochrome P450 CYP71	9.85
1.0	0.0	0.0	0.0	Contig_10649	UDP-glucosyltransferase	7.07
1.0	0.4	0.1	0.2	Contig_31446	Cytochrome P450 CYP83	2.19
1.0	0.0	0.2	0.2	Contig_2707	Cytochrome P450 CYP72	2.69
1.0	0.3	0.0	0.3	Contig_2935	Cytochrome P450 CYP71	2.33
1.0	0.2	0.3	0.2	Contig_6935	Indole-3-glycerol-phosphate lyase	2.14
1.0	0.0	0.3	0.0	Contig_30857	Dioxygenase	3.14
1.0	1.0	0.2	0.3	Contig_32831	UDP-glucosyltransferase	1.02
1.0	0.1	0.1	0.2	Contig_19787	Dioxygenase	2.55
1.0	0.2	0.2	0.4	Contig_5376	UDP-glucosyltransferase	1.92
1.0	0.3	0.3	0.3	Contig_1708	Cytochrome P450 CYP76	1.65
1.0	0.2	0.1	0.0	Contig_712	Flavin-containing monooxygenase	3.20
1.0	0.1	0.5	0.2	Contig_5450	Cytochrome P450 CYP714	2.01
1.0	0.0	0.1	0.8	Contig_14493	Cytochrome P450 CYP83	1.76
1.0	0.5	0.3	0.1	Contig_2780	Cytochrome P450 CYP82	1.86
0.9	1.0	0.3	0.0	Contig_11857	Cytochrome P450 CYP79	1.09
1.0	0.2	0.1	0.0	Contig_30397	Cytochrome P450 CYP76	3.02
1.0	0.3	0.3	0.4	Contig_8970	2-oxoglutarate-dependent dioxygenase	1.56
1.0	0.1	0.4	0.0	Contig_11225	Cytochrome P450 CYP76	2.51
1.0	0.4	0.6	0.2	Contig_11634	UDP-glucosyltransferase	1.37
1.0	0.1	0.1	0.0	Contig_15518	UDP-glucosyltransferase	3.79
1.0	0.4	0.0	0.0	Contig_11628	UDP-glucosyltransferase	2.86
1.0	0.6	0.5	0.2	Contig_22511	Cytochrome P450 CYP78	1.20
1.0	0.0	0.0	0.0	Contig_17353	Cytochrome P450 CYP716	7.37
1.0	0.5	0.6	0.3	Contig_22248	UDP-glucosyltransferase	1.21
1.0	0.5	0.1	0.4	Contig_830	UDP-glucosyltransferase	1.76
1.0	1.0	0.4	0.1	Contig_9571	Cytochrome P450 CYP96	1.00
1.0	0.6	0.0	0.0	Contig_20947	Cytochrome P450 CYP76	2.20
1.0	0.4	0.1	0.9	Contig_675	Cytochrome P450 CYP75	1.12
1.0	0.2	0.4	0.5	Contig_31681	Cytochrome P450 CYP75	1.62
1.0	0.1	0.1	0.0	Contig_27203	Cytochrome P450 CYP76	3.93
0.9	0.0	0.0	1.0	Contig_6474	Cytochrome P450 CYP704	1.47
1.0	0.0	0.0	0.0	Contig_32789	Cytochrome P450 CYP716	5.80
1.0	0.0	0.0	0.0	Contig_23988	Cytochrome P450 CYP76	9.33
1.0	0.1	0.0	0.0	Contig_18314	Cytochrome P450 CYP87	3.95
1.0	0.4	0.4	0.1	Contig_1641	Cytochrome P450 CYP78	1.70
1.0	0.1	0.5	0.6	Contig_4024	Cytochrome P450 CYP71	1.23
1.0	0.0	0.1	0.0	Contig_5757	Cytochrome P450 CYP72	5.82
1.0	0.2	0.1	0.0	Contig_10832	UDP-glucosyltransferase	3.60
1.0	0.7	0.7	0.0	Contig_21178	Dioxygenase	1.03
1.0	0.3	0.4	0.4	Contig_4269	Cytochrome P450 CYP72	1.41
1.0	0.3	0.1	0.0	Contig_30050	Cytochrome P450 CYP75	2.66
1.0	0.1	0.1	0.0	Contig_23142	Cytochrome P450 CYP71	3.46
1.0	0.1	0.3	0.0	Contig_15395	Cytochrome P450 CYP736	2.68
1.0	0.4	0.1	0.1	Contig_14423	UDP-glucosyltransferase	2.16
1.0	0.8	0.2	0.3	Contig_10495	Cytochrome P450 CYP734	1.20
1.0	0.2	0.2	0.5	Contig_27454	Cytochrome P450 CYP71	1.86
1.0	0.5	0.0	0.0	Contig_19157	Cytochrome P450 CYP87	2.61
1.0	0.1	0.2	0.5	Contig_403	Indole-3-glycerol-phosphate lyase	1.85

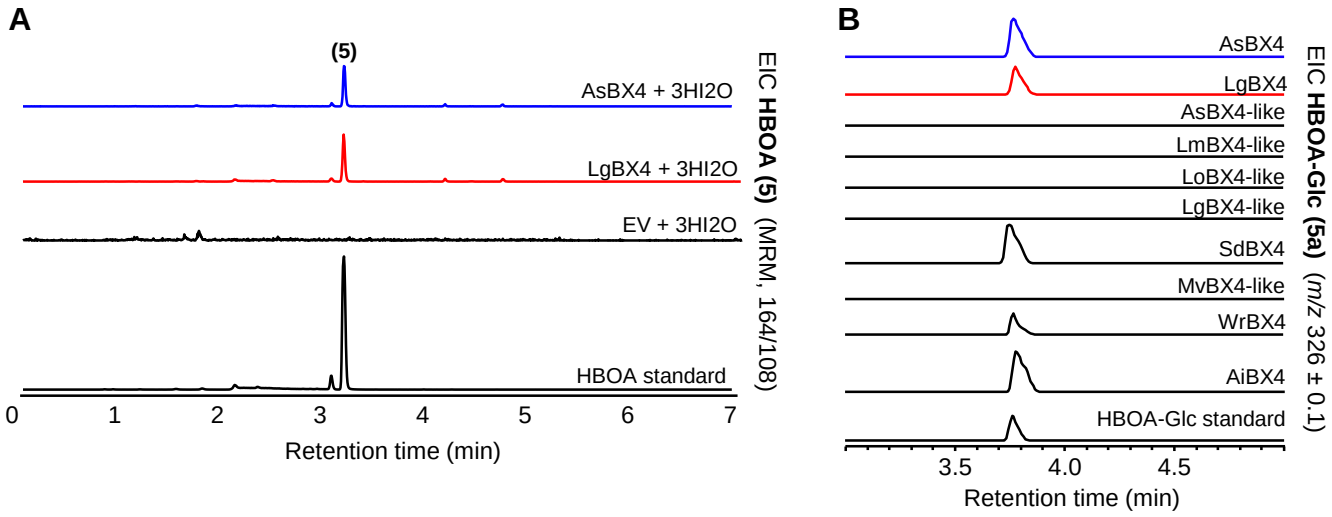
SI Figure 2: Complete list of BXD gene candidates from the transcriptomic analysis of *A. squarrosa* and *L. galeobdolon* A) 90 candidates were retrieved from *A. squarrosa* transcriptomic analysis comprising cytochrome P450s, 2-oxoglutarate-dependent dioxygenases, flavin-containing monooxygenases, polyphenol oxidases, O-methyltransferases, and UDP-glycosyltransferases. B) 57 candidates were retrieved from *L. galeobdolon* including cytochrome P450s, 2-oxoglutarate-dependent dioxygenases, flavin-containing monooxygenases, and UDP-glycosyltransferases. Values from 0 (lowest) to 1 (highest), indicate the relative expression of each gene among the tested conditions.



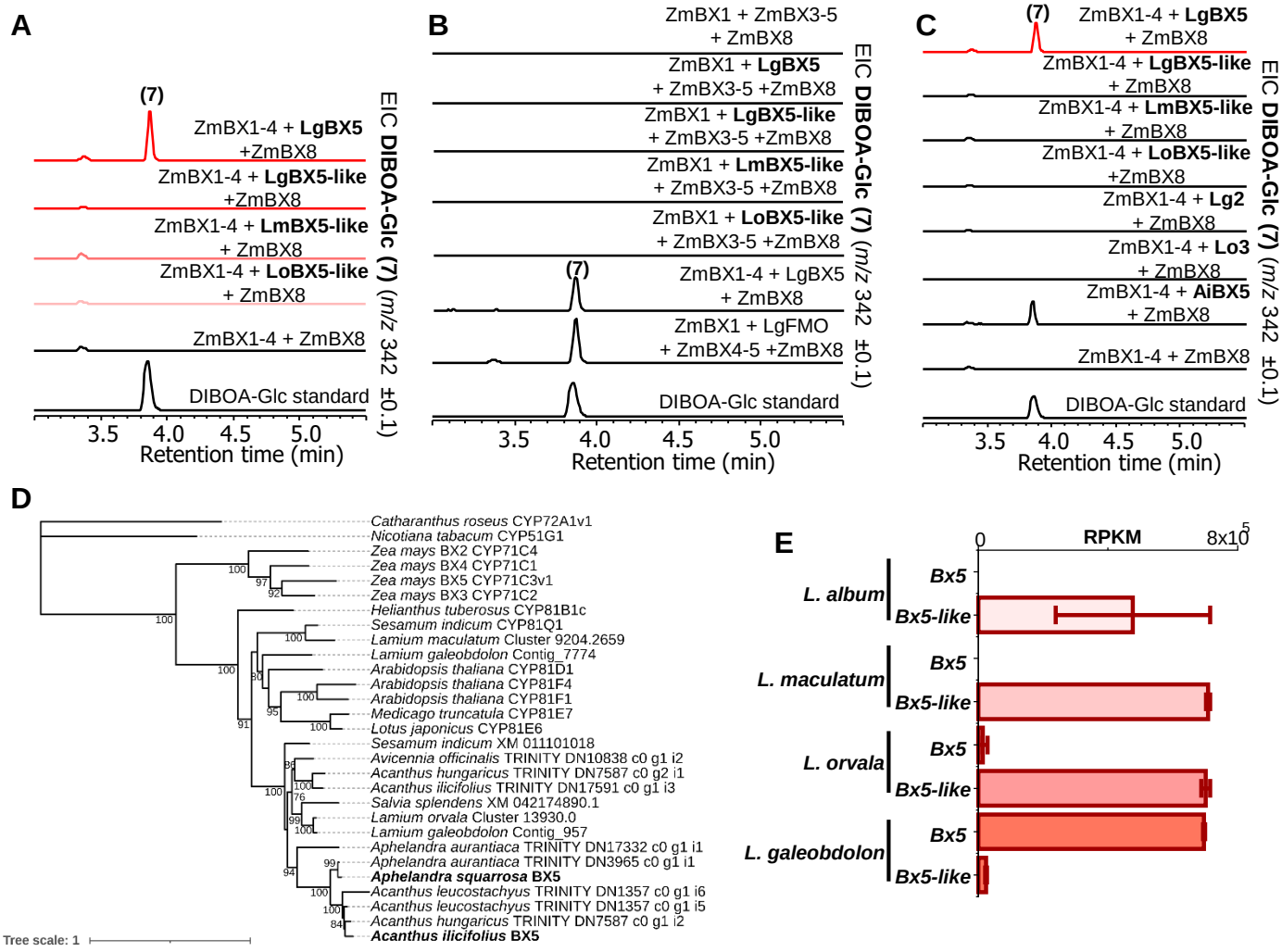
SI Figure 3: Establishing *Nicotiana benthamiana* as a platform for candidate gene screening. Combinatorial reconstitution of the *Zea mays* BXD pathway by *Agrobacterium tumefaciens*-mediated transient transformation of *N. benthamiana*. Different combinations of the maize *Bx* genes were expressed and LC-qTOF traces of leaf methanol extracts are displayed as extracted ion chromatograms for I2O (3) (M+H, *m/z* 134 ± 0.1), 3HI2O-Glc (4a, 4b) (M-H, *m/z* 310 ± 0.1), HBOA-Glc (5a) (M-H, *m/z* 326 ± 0.1), DIBOA-Glc (7) (M-H, *m/z* 342 ± 0.1), TRIBOA-Glc (8) (M+H, *m/z* 360 ± 0.1), and DIMBOA-Glc (9) (M-H, *m/z* 372 ± 0.1). We hypothesize that the two peaks detected for 3HI2O-Glc represent two isomers of 3HI2O-Glc (4a, 4b). The isomers were not characterized but the possible structures are shown. Compounds are numbered according to Fig. 1A.



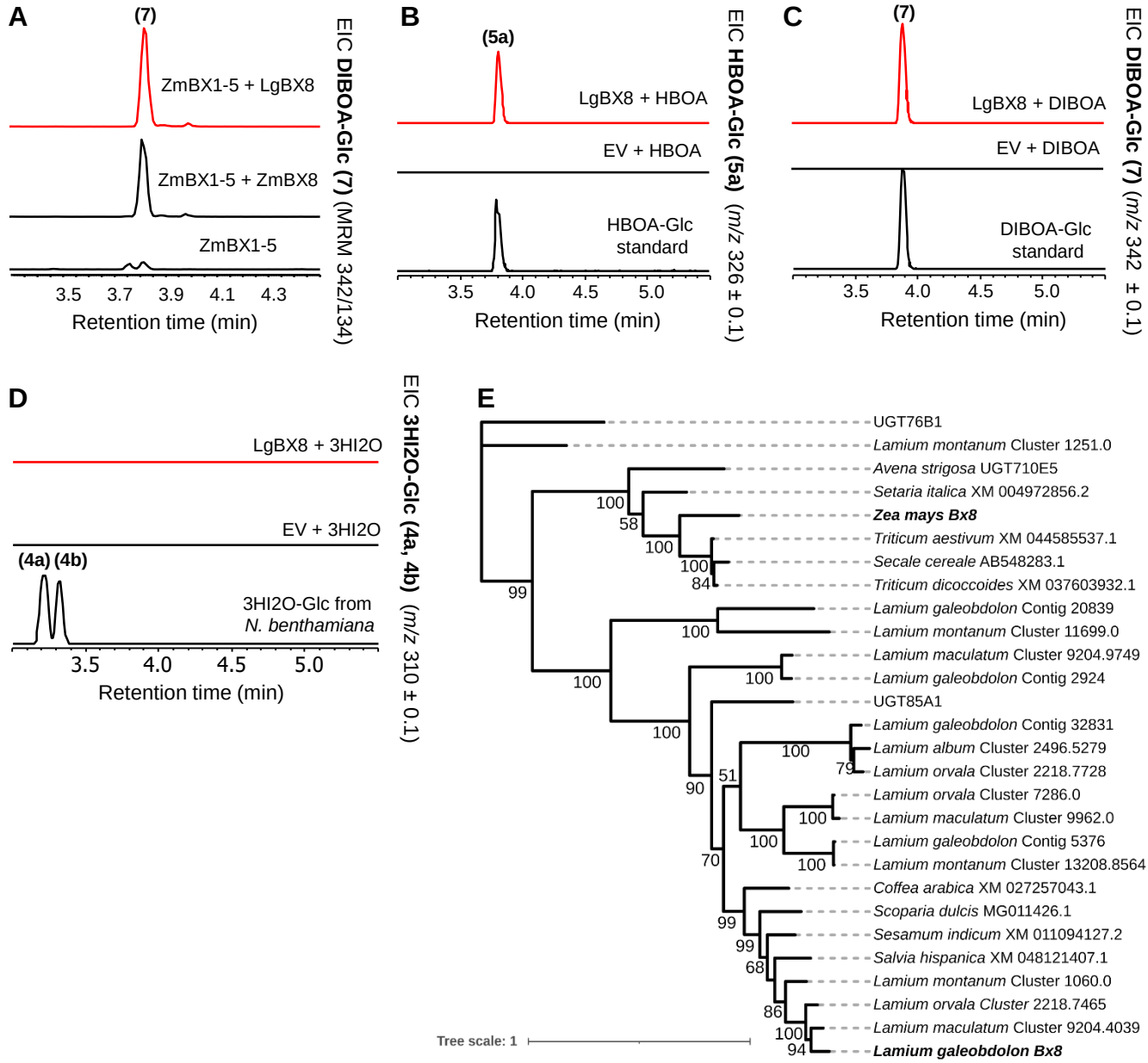
SI Figure 4: FMOs related to AsFMO and LgFMO from several dicot families display BX2/BX3 activity. A) Candidate FMO genes were transiently expressed with *ZmBx1* and *ZmBx8* in *N. benthamiana* and leaf methanol extracts were analyzed using LC-qTOF. AsFMO (*Aphelandra squarrosa*), LgFMO (*Lamium galeobdolon*), SiFMO (*Sesamum indicum* XM011080249.2), Lc (*Leonurus cardiac* c33258_g1_i2), Pf (*Phlomis fruticosa* c68310_g1_i1), Lm (*Lamium maculatum* Cluster-9204.5432), La (*Lamium album* Cluster-2496.6023), Mv (*Marrubium vulgare* c26472_g1_i2), Ro (*Rosmarinus officinalis* c40410_g1_i1), Ai (*Acanthus ilicifolius* TRINITY DN1050 c0 g1 i3). EIC, extracted ion chromatogram. B) AsFMO and LgFMO use both indole (2) and I2O (3) as substrate for 3HI2O-Glc (4a, 4b) formation. *AsFMO* and *LgFMO* were transiently expressed in *N. benthamiana* and leaves were infiltrated with indole (2) or I2O (3) as FMO substrates. Leaf methanol extracts were analyzed using LC-qTOF. Means ± SE (n = 3) are shown.



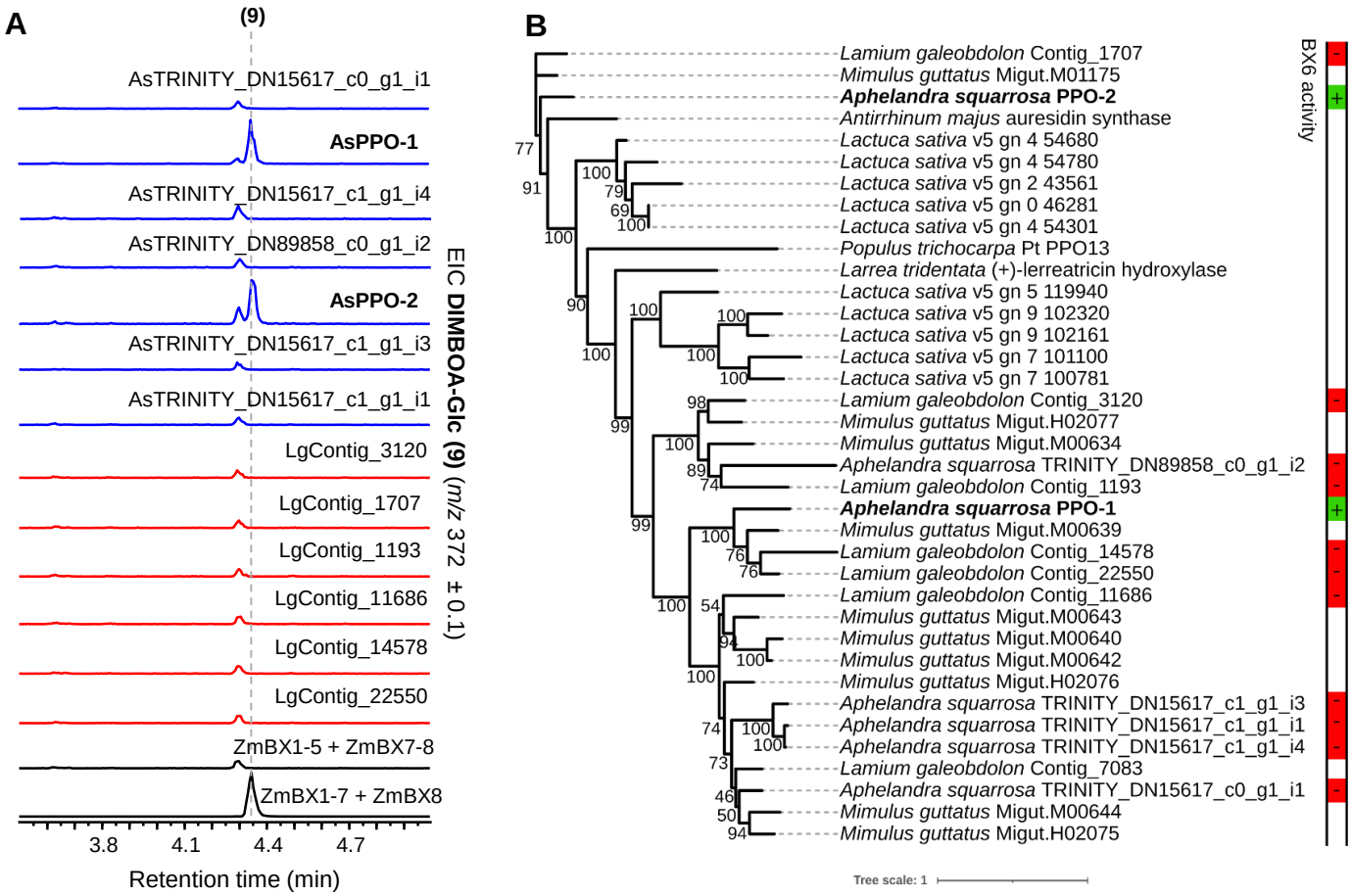
SI Figure 5: Microsome preparations of *AsBx4*- or *LgBx4*-expressing *Saccharomyces cerevisiae* showed conversion of 3HI2O (4) to HBOA (5). A) *AsBx4* and *LgBx4* were expressed in *S. cerevisiae* and yeast microsomes were incubated with 3HI2O (4) and NADPH. Enzyme products were analyzed using LC-MS/MS (MRM). HBOA formation was confirmed with an authentic standard. EIC, extracted ion chromatogram; EV, empty vector control. B) For testing potential BX4 activity of BX4-like enzymes from different eudicot species, the respective gene was transiently coexpressed with maize *ZmBx1-3* and *ZmBx8* in *N. benthamiana*. Leaf methanol extracts were analyzed using LC-qTOF. AsBX4-like (*A. squarrosa* TRINITY_DN4049_c0_g1_i2), LmBX4-like (*L. maculatum* Cluster-9204.7837), LoBX4-like (*L. orvala* Cluster-2218.4195), LgBX4-like (*L. galeobdolon* TRINITY_DN9871_c0_g1_i7), SdBX4 (*Scoparia dulcis* CYP92A46), MvBX4-like (*Marrubium vulgare* c20842_g1_i1), WrBX4 (*Wrightia religiosa* TRINITY_DN979_c0_g1_i1), AiBX4 (*Acanthus ilicifolius* TRINITY_DN9057_c0_g1_i13).



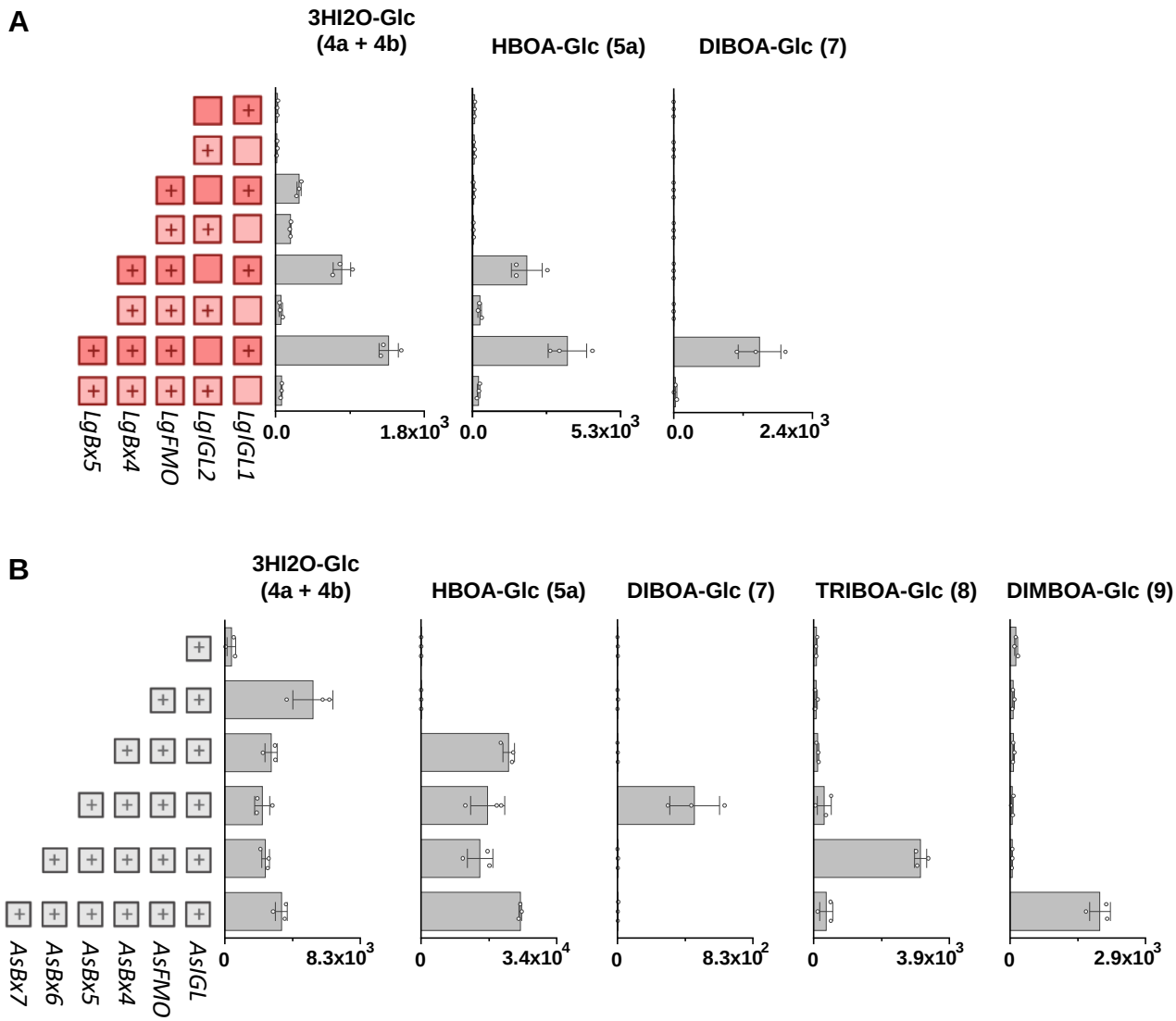
SI Figure 6: Characterization of BX5 and BX5-like proteins from Lamiaceae and Acanthaceae species. A) BX5-like from different *Lamium sp.* show no BX5 activity in *N. benthamiana*. *N. benthamiana* was transiently transformed with *ZmBx1*, *ZmBx2*, *ZmBx3*, *ZmBx4*, *ZmBx8*, and *L. galeobdolon*, *L. maculatum,* or *L. orvala Bx5-like* and methanol extracts of treated leaves were analyzed using LC-qTOF. EIC, extracted ion chromatogram. B) BX5-like from different *Lamium sp.* show no BX2 activity in *N. benthamiana*. LC-qTOF traces of methanolic extracts of *N. benthamiana* transiently transformed with *ZmBx1, ZmBx3, ZmBx4, ZmBx5, ZmBx8*, and *Bx5-like* from *L. galeobdolon*, *L. maculatum,* and *L.orvala* are shown. LgFMO was included as positive control. C) LC-qTOF analysis of methanol extracts of *N. benthamiana* leaves transiently expressing sequences close to *LgBx5* along with *ZmBx1, ZmBx2, ZmBx3, ZmBx4*, and *ZmBx8*. Sequence abbreviations: LgBX5 (*L. galeobdolon* BX5), LgBX5-like (*L. galeobdolon* BX5-like), LoBX5 (*L. orvala* BX5-like), Lg2 (*L. galobdolon* TRINITY DN4514 c0 g1 i3), Lo3 (*L. orvala* Cluster 10786.0), AiBX5 (*Acanthus ilicifolius* BX5). D) BX5 activity has likely a monophyletic origin in the Acanthaceae. *A. ilicifolius* BX5, which showed BX5 activity (SI Fig. 6C), clusters together with AsBX5. E) Expression of *Lamium Bx5 and Bx5-like* genes in mature leaves of different *Lamium sp.*. Expression was analyzed by RNAseq (n = 3 biological replicates ± SE) and RPKM values are given. F) Comparative activity of Lamium BX5 and ZmBX2 microsome preparation. Microsome preparations were incubated with indole and NADPH for 2h. I2O formation was measured trough targeted LC-MS. n = 4, SE is displayed.



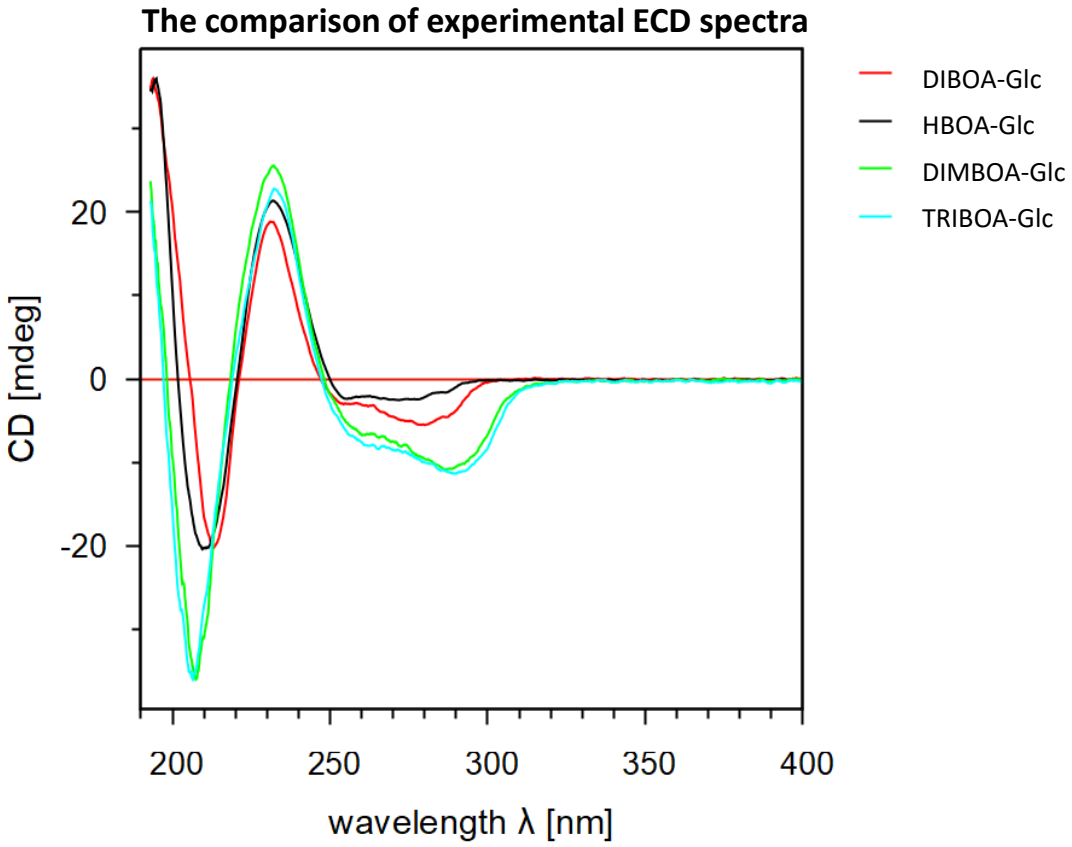
SI Figure 7: *In vivo* and *in vitro* characterization and phylogenetic analysis of LgBX8. A) *LgBx8* was expressed with *ZmBx1-5* in *Nicotiana benthamiana* and leaf extracts were analyzed using LC-MS/MS (MRM). B, C and D) Purified LgBX8 heterologously expressed in *Escherichia coli* was incubated with the potential substrates HBOA (5) (B), DIBOA (6) (C) and 3HI2O (4) (D). Enzyme products were analyzed using LC-qTOF. Accumulation of HBOA-Glc (5a) and DIBOA-Glc (7) was confirmed with authentic standards. In absence of 3HI2O-Glc (4a, 4b) authentic standard, 3HI2O-Glc (4a, 4b) traces from *N. benthamiana* ZmBX1-BX3 reconstitution were used as putative standards. EIC, extracted ion chromatogram. EV, empty vector control. E) Maximum likelihood phylogenetic analysis of LgBX5, ZmBX5, and related proteins from monocot and Lamiales species.



SI Figure 8: Two polyphenol oxidases (PPOs) from *Aphelandra squarrosa* have BX6 activity. A) Candidate genes were transiently expressed with maize *ZmBx1-5* + *ZmBx7-8* in *N. benthamiana* and methanolic leaf extracts were analyzed using LC-qTOF to measure DIMBOA-Glc accumulation (9). EIC, extracted ion chromatogram. B) Maximum likelihood phylogenetic analysis of *A. squarrosa* and *L. galeobdolon* PPOs that were assessed for BX6 activity (green (+) = active, red (-) = inactive).



SI Figure 9: Expression of *LgBx* and *AsBx* genes, respectively, in *Nicotiana benthamiana* lead to the functional reconstitution of the *Aphelandra squarrosa* and *Lamium galeobdolon* BXD pathways. *L. galeobdolon Bx* genes (A) and *A. squarrosa Bx* genes (B), respectively, were transiently expressed in leaves of *N. benthamiana* and leaf methanol extracts were analyzed using LC-qTOF. Squares containing a '+' symbol indicate the presence of the respective gene in the transformation mixture. All combinations of *Lamium Bx* genes tested contained *LgBx8*, while *Aphelandra Bx* genes were always co-expressed with maize *ZmBx8*. Shown are averaged peak areas / mg fresh weight ± SE (n = 3 biological replicates).



SI Figure 10: Comparison of experimental ECD spectra for HBOA-Glc, DIBOA-Glc, TRIBOA-Glc, and DIMBOA-Glc. (*2R*)-DIBOA-Glc and (*2R*)-DIMBOA-Glc exhibited positive Cotton effects around 230 nm (Nagao *et al.,*1985). A positive Cotton effect at 228 nm and a negative at 291 nm have been observed in (*2R*)-DIMBOA-Glc (Hartenstein *et al.,* 1993)). The optical rotation for DIBOA-Glc was determined as $[a]_D^{20}$ +66.1 (c 0.47, H_2O). This is in accordance with literature values for the (2*R*)-isomer (Hartenstein *et al.,* 1994)). We therefore concluded TRIBOA to have also a (2*R*)-configuration.

Nagao T, Otsuka H, Kohda H, Sato T, Yamasaki K (1985) Benzoxazinones from *Coix lachryma-jobi* var. ma-yuen. *Phytochemistry* 24: 2959-2962

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Hartenstein H, Sicker D (1994) (2*R*)-2-b-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one from *Secale cereale*. *Phytochemistry* 35: 827-828

Supplemental Table 1: Percentages of amino acid sequence identities between tested enzymes. The tables display the percentage of AA sequence identity between enzymes used in phylogenetic inference. Sequences were aligned with MUSCLE 5.1. The results are reported for FMOs (A), BX4 (B), BX5 (C), BX6 (D), BX7 (E), and BX8 (F). Green or red coloring, respectively, indicates presence or absence of the activity indicated in the title of the table.

A Percentage of AA seq identity among functionally tested FMOs

	Achantus ilicifolius TRINITY_DN1050_c0_g1_i3	Aphelandra squarrosa Bx2/Bx3	Lamium album Cluster-2496.6023	Lamium galeobdolon Contig_33225	Lamium galeobdolon Bx2/Bx3	Lamium maculatum Cluster-9204.5432	Leonurus cardiaca c33258_g1_i2	Marrubium vulgare c26472_g1_i2	Phlomis fruticosa c68310_g1_i1	Rosmarinus officinalis c40410_g1_i1	Sesamum indicum XM_011080249.2
Achantus ilicifolius TRINITY_DN1050_c0_g1_i3		81.4	60.7	62.1	63.7	59.9	61.2	64.4	59.2	60.1	67.6
Aphelandra squarrosa_Bx2/Bx3	81.4		62.4	63.2	65	61.7	64.2	67.3	61.5	63.1	70.4
Lamium album Cluster-2496.6023	60.7	62.4		88.5	87.1	72.1	83.2	74	81.3	68.6	72.3
Lamium galeobdolon Contig_33225	62.1	63.2	88.5		89.2	72.4	84.1	75.8	79.7	69.7	74.7
Lamium galeobdolon_Bx2/Bx3	63.7	65	87.1	89.2		73.2	82.7	74.9	77.5	68.9	72.6
Lamium maculatum Cluster-9204.5432	59.9	61.7	72.1	72.4	73.2		71.6	70.9	68.4	65.6	70.9
Leonurus cardiaca c33258_g1_i2	61.2	64.2	83.2	84.1	82.7	71.6		75.9	81.9	68.9	74.1
Marrubium vulgare c26472_g1_i2	64.4	67.3	74	75.8	74.9	70.9	75.9		73.1	71	75.8
Phlomis fruticosa c68310_g1_i1	59.2	61.5	81.3	79.7	77.5	68.4	81.9	73.1		67.3	72.7
Rosmarinus officinalis c40410_g1_i1	60.1	63.1	68.6	69.7	68.9	65.6	68.9	71	67.3		69.6
Sesamum indicum XM_011080249.2	67.6	70.4	72.3	74.7	72.6	70.9	74.1	75.8	72.7	69.6	

B Percentage of AA seq identity among functionally tested BX4

	Achantus ilicifolius TRINITY_DN9057_c0_g1_i13	Aphelandra squarrosa BX4	Aphelandra squarrosa TRINITY DN4049 c0 g1 i2	Lamium galeobdolon Contig_675	Lamium galeobdolon BX4	Lamium maculatum Cluster-9204.7837	Lamium orvala Cluster-2218.4195	Marrubium vulgare c20842_g1_i1	Scoparia dulcis CYP92A46	Wrightia_religiosa scaffold_2074295	Zea mays BX4
Achantus ilicifolius TRINITY_DN9057_c0_g1_i13		83.9	59.6	57.1	60	56.4	59.5	52	57.1	53.2	30.2
Aphelandra squarrosa BX4	83.9		59.4	57	59.5	57.1	58.2	51.2	55.8	52.5	31.4
Aphelandra squarrosa TRINITY DN4049 c0 g1 i2	59.6	59.4		59.2	60	61.9	60.6	54	55.3	52.9	29.3
Lamium galeobdolon Contig_675	57.1	57	59.2		79.1	79.3	81.5	60.7	57.2	55.5	29.2
Lamium galeobdolon BX4	60	59.5	60	79.1		85.7	86.2	61	60.5	56.7	29.3
Lamium maculatum Cluster-9204.7837	56.4	57.1	61.9	79.3	85.7		88.9	60.1	59.1	57.3	28.9
Lamium orvala Cluster-2218.4195	59.5	58.2	60.6	81.5	86.2	88.9		60.6	59	56.7	30
Marrubium vulgare c20842_g1_i1	52	51.2	54	60.7	61	60.1	60.6		55.8	55	28.6
Scoparia dulcis CYP92A46	57.1	55.8	55.3	57.2	60.5	59.1	59	55.8		54.8	29.9
Wrightia_religiosa scaffold_2074295	53.2	52.5	52.9	55.5	56.7	57.3	56.7	55	54.8		30.8
Zea mays BX4	30.2	31.4	29.3	29.2	29.3	28.9	30	28.6	29.9	30.8	

C Percentage of AA seq identity among functionally tested BX5

	Acanthus ilicifolius BX5	Aphelandra squarrosa BX5	Lamium galeobdolon BX5	Lamium galeobdolon BX5-like	Lamium galeobdolon Contig_32188	Lamium maculatum BX5-like	Lamium orvala BX5-like	Lamium orvala Cluster-10786.0	Zea mays BX5
Acanthus ilicifolius BX5		77.5	28.7	28.8	30.9	28.2	28	26.8	25.2
Aphelandra squarrosa BX5	77.5		29.5	29.1	29.4	28.5	28.5	28.4	25.6
Lamium galeobdolon BX5	28.7	29.5		87.1	60.6	84.5	85.3	68.1	32.9
Lamium galeobdolon BX5-like	28.8	29.1	87.1		65.7	93.5	95	72.6	33.3
Lamium galeobdolon Contig_32188	30.9	29.4	60.6	65.7		64.9	65.1	62.4	32
Lamium maculatum BX5-like	28.2	28.5	84.5	93.5	64.9		95.4	71.6	32.8
Lamium orvala BX5-like	28	28.5	85.3	95	65.1	95.4		70.8	32.6
Lamium orvala Cluster-10786.0	26.8	28.4	68.1	72.6	62.4	71.6	70.8		32.5
Zea mays BX5	25.2	25.6	32.9	33.3	32	32.8	32.6	32.5	

D Percentage of AA seq identity among functionally tested BX6

	Aphelandra squarrosa BX6	Zea mays BX6
Aphelandra squarrosa BX6		29.6
Zea mays BX6	29.6	

E Percentage of AA seq identity among functionally tested BX7

	Aphelandra squarrosa BX7	Zea mays BX7
Aphelandra squarrosa BX7		27.5
Zea mays BX7	27.5	

F Percentage of AA seq identity among functionally tested BX8

	Lamium galeobdolon BX8	Zea mays BX8
Lamium galeobdolon BX8		31.4
Zea mays BX8	31.4	

Supplemental Table 2: Primers used in this study.

Accession	Species	Forward primer	Reverse primer	Vector	Host
TRINITY_DN10074_c0_g1_i1	*Aphelandra squarrosa*	ATATAGCGGCCGCAATGTCGCCTACCTTGATCTTG	TTAATTAATCACATATAAGGTTTGGCAATC	pCambia2300	*N. benthamiana*
TRINITY_DN10074_c0_g1_i2	*Aphelandra squarrosa*	ATATAGCGGCCGCAATGGAATTCGAATTTCCTTTCAAC	TTAATTAATCACATATAAGGTTTGGCAATC	pCambia2300	*N. benthamiana*
TRINITY_DN5474_c0_g1_i7	*Aphelandra squarrosa*	ATATAGCGGCCGCAATGGAGATCATCCAGTTGTTG	TTAATTAATTAGGTCACCGGTCTTTTAATC	pCambia2300	*N. benthamiana*
TRINITY_DN2050_c0_g1_i5	*Aphelandra squarrosa*	TTATGAATTTTGCAGATGGAACTGCTGACCAGCTCA	GACAACCACAACAAGCACCGTCAATAGAGGTGCGGGGATAGTCT	3Ω1	*N. benthamiana*
TRINITY_DN1895_c0_g1_i6	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGATACTGTCCATCACTGTAG	GACAACCACAACAAGCACCGTTAAATTCGTTCCAAGATTAACTGAG	3Ω1	*N. benthamiana*
TRINITY_DN2050_c0_g1_i6	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAAGTGGTGACCAGCTC	GACAACCACAACAAGCACCGCTAATAGAGATGAGAAGAGAGC	3Ω1	*N. benthamiana*
TRINITY_DN2050_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAACTGCTGACCAGCTC	GACAACCACAACAAGCACCGTTAATAGAGGTGCGGGGAGAG	3Ω1	*N. benthamiana*
TRINITY_DN4480_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCCAAAGTCTGTAACCAAATTC	GACAACCACAACAAGCACCGTTAGTAAAGGTGGGGTGGGAG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCATGGCCAAAGTCTGTAACCAAATTC	CTCTGGCGAAGAATTGTTAATTTAGTAAAGGTGGGGTGGGAG	pESC-Leu	*S. cerevisiae*
TRINITY_DN6380_c0_g1_i11	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAATCTTCCACAGTTGGCC	GACAACCACAACAAGCACCGTCAATGGTAGAGCTTTGGATTGG	3Ω1	*N. benthamiana*
TRINITY_DN5373_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGATGCTCGAAACACCCCTAC	GACAACCACAACAAGCACCGCTAATTCGGCAACGAAGCCAC	3Ω1	*N. benthamiana*
TRINITY_DN1999_c0_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCCTGGATTTGGACGG	GACAACCACAACAAGCACCGTTAATTGCACAACCGATAGTTAGGAAT	3Ω1	*N. benthamiana*
TRINITY_DN3965_c0_g1_i1	*Aphelandra squarrosa*	TTATGAATTTTGCAGATGGACGTGTCAATAACCGAAATC	GACAACCACAACAAGCACCGTCAGGCAATGACGGTAGCC	3Ω1	*N. benthamiana*
TRINITY_DN990_c0_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGGTGGTCTCTTGCCCA	GACAACCACAACAAGCACCGCTACAGATGATGCAGAACAACG	3Ω1	*N. benthamiana*
TRINITY_DN2021_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGGTCTCGCACCTG	GACAACCACAACAAGCACCGTCACTTAATTTTCCGCATGGTTAAG	3Ω1	*N. benthamiana*
TRINITY_DN3629_c1_g2_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGTGCATTGTCATTAAAACACACAG	GACAACCACAACAAGCACCGCTAAACAGGGGCAGTCTTAG	3Ω1	*N. benthamiana*
TRINITY_DN5057_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTATCTTCTTCTTCCTCC	GACAACCACAACAAGCACCGTTACATGTCGACAGCCAAACGTTTG	3Ω1	*N. benthamiana*
TRINITY_DN14457_c0_g1_i3	*Aphelandra squarrosa*	TTATGAATTTTGCAGATGGAGAACCCACTGCTG	GACAACCACAACAAGCACCGTTATGAGTTGTGGAGTATGG	3Ω1	*N. benthamiana*
TRINITY_DN2569_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAAGGCAGTCTGCTGTACAC	GACAACCACAACAAGCACCGCTAATCCACATTATCTGATTCTTGAATAAC	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCATGGAAGGCAGTCTGCTGTACAC	CTCTGGCGAAGAATTGTTAATCTAATCCACATTATCTGATTCTTGAATAAC	pESC-Leu	*S. cerevisiae*
TRINITY_DN4049_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGTTCATGAACATGGTGGACTTC	GACAACCACAACAAGCACCGTCAGTAAAGGTGAGGTGGAAGTG	3Ω1	*N. benthamiana*
TRINITY_DN37698_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGACGTGTGGAGCATCAAAC	GACAACCACAACAAGCACCGTCACAAGGAATACATTTCAAGTGAAAG	3Ω1	*N. benthamiana*

TRINITY_DN84281_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAGCGCTGCAGCCTTGATC	GACAACCACAACAAGCACCGTCACGGCTGAGTTTGGTAGAC	3Ω1	*N. benthamiana*
TRINITY_DN3189_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGATTCTCACCCACTACC	GACAACCACAACAAGCACCGTCAAAGCTGTGACAGAAGATGGC	3Ω1	*N. benthamiana*
TRINITY_DN6845_c0_g1_i8	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGATCCATCTACCTTCAAACT	GACAACCACAACAAGCACCGTCAGTCCAAAGACTTGTAGACAAC	3Ω1	*N. benthamiana*
TRINITY_DN1895_c0_g1_i9	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGATACTGTCCATCACTGTAG	GACAACCACAACAAGCACCGTTAAATTCGTTCCAAGATTAACTGAGC	3Ω1	*N. benthamiana*
TRINITY_DN4349_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTGCCGCTAACTCTCTC	GACAACCACAACAAGCACCGTTAGAAGCCGTTAGAAGCCGG	3Ω1	*N. benthamiana*
TRINITY_DN5746_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGTCTTCATATTTGGACAAGGCTTC	GACAACCACAACAAGCACCGTCATTTAGAATGAGCGATCAAATTAG	3Ω1	*N. benthamiana*
TRINITY_DN3189_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGATTCTCACCCACTACC	GACAACCACAACAAGCACCGTCAAAGCTGTGACAGAAGATGGC	3Ω1	*N. benthamiana*
TRINITY_DN476_c0_g1_i6	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGATGAGGTTGGTTTCAGTTGG	GACAACCACAACAAGCACCGCTACTTTTGGTACATTGATGCAGAC	3Ω1	*N. benthamiana*
TRINITY_DN16988_c0_g1_i5	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATGGTTACCAACAGCTGATC	GACAACCACAACAAGCACCGTTAATTCTCATATGAGGTAACCGACAC	3Ω1	*N. benthamiana*
TRINITY_DN9249_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCACCTGAATTCTCCCTC	GACAACCACAACAAGCACCGTTAACCTCGCCTGTCTCGG	3Ω1	*N. benthamiana*
TRINITY_DN20009_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGTTCGTCTATATAACAAGTTTATCC	GACAACCACAACAAGCACCGCTAATTCACCAATGTAGCCACCAAG	3Ω1	*N. benthamiana*
TRINITY_DN137500_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCACCCACTGTTACAACTTC	GACAACCACAACAAGCACCGTCATATCCTAAGCAAAGAAAGAGCC	3Ω1	*N. benthamiana*
TRINITY_DN4513_c0_g1_i5	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATCTTCTCCTCGTCGAG	GACAACCACAACAAGCACCGTTATTTTCGGGCTGAAGATCTTGG	3Ω1	*N. benthamiana*
TRINITY_DN2050_c0_g1_i4	*Aphelandra squarrosa*	ATGGAAGTGGTGACCAGC	TTAATAGAGTTGAGAAGAGAGCCT	pCambia2300	*N. benthamiana*
TRINITY_DN14367_c0_g1_i2	*Aphelandra squarrosa*	ATGTATAATTCAGTCAAGGATGATC	TTATGGCGGCAAGGGA	pCambia2300	*N. benthamiana*
TRINITY_DN476_c0_g1_i8	*Aphelandra squarrosa*	ATGATCATCCCTTCAGATCTATT	TTAGCGAGTTTGGTACATTGA	pCambia2300	*N. benthamiana*
TRINITY_DN5474_c0_g1_i4	*Aphelandra squarrosa*	ATGCCAATGGAGATCATCC	TCACCAATGAATTGCATCG	pCambia2300	*N. benthamiana*
TRINITY_DN4025_c0_g1_i2	*Aphelandra squarrosa*	ATGGAGATCCAGCTACCTT	TTAATTGGAAGCGTTGTAGAG	pCambia2300	*N. benthamiana*
TRINITY_DN4265_c0_g1_i1	*Aphelandra squarrosa*	ATGGAAATTATTGGCATGGAG	TCAGTTGACATGAGTGAGAAG	pCambia2300	*N. benthamiana*
TRINITY_DN28850_c0_g1_i2	*Aphelandra squarrosa*	ATGGAAACTTTGTTGGTTCTC	CTAAAGGTTGCGGAGAATGA	pCambia2300	*N. benthamiana*
TRINITY_DN33184_c0_g1_i1	*Aphelandra squarrosa*	ATGGAAGCAAAACTAGGGT	TCAGGATATAGACAGTGGTGG	pCambia2300	*N. benthamiana*
TRINITY_DN11835_c0_g1_i1	*Aphelandra squarrosa*	ATGGACAATGTAGTAATGTTGTT	TTATTTAAGCGGCGGCA	pCambia2300	*N. benthamiana*
TRINITY_DN7413_c0_g2_i3	*Aphelandra squarrosa*	ATGGATTTGTGGGTGGTG	TCAAACCCCATCTCCGG	pCambia2300	*N. benthamiana*
TRINITY_DN111532_c0_g1_i1	*Aphelandra squarrosa*	ATGATCTCTATTGCAATATGCAT	TCACACCCATCTACTCAAAA	pCambia2300	*N. benthamiana*
TRINITY_DN5547_c0_g1_i1	*Aphelandra squarrosa*	ATGTCCAAATTACATCTGTTATTACA	TCAACAATGATGATCAGAAGC	pCambia2300	*N. benthamiana*
TRINITY_DN151_c1_g1_i1	*Aphelandra squarrosa*	ATGAGCTTACTAGAAATCGTGA	TTAAGATGGGGTGGCATATC	pCambia2300	*N. benthamiana*

TRINITY_DN151_c1_g1_i1	*Aphelandra squarrosa*	ATGGCAACTGCCTTTCA	TTAAATCAAAGCCGGCATAAG	pCambia2300	*N. benthamiana*
TRINITY_DN36592_c0_g1_i1	*Aphelandra squarrosa*	ATGGAGTTTCACTACATCCTC	CTAACTCAATTGGACAACCC	pCambia2300	*N. benthamiana*
TRINITY_DN8666_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGCACACCAAATCCCTCTTCTT	GACAACCACAACAAGCACCGCTAGTCAGCCTTTTGGGTAAAC	3Ω1	*N. benthamiana*
TRINITY_DN8666_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGCACACCAAATCCCTCTTCTTTAT	GACAACCACAACAAGCACCGCTAGTCAGCCTTTTGGGTAAACTT	3Ω1	*N. benthamiana*
TRINITY_DN6556_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGCAGATGAAGAAAACGGTGG	GACAACCACAACAAGCACCGTCACTTGGCAAAATAACTGGGGTTG	3Ω1	*N. benthamiana*
		AAGTTCTGTTTCAGGGCCCGCAGATGAAGAAAACGGTGG	ATGGTCTAGAAAGCTTTACTTGGCAAAATAACTGGGGTTG	pOPINF	*E. coli*
		AAGTTCTGTTTCAGGGCCCGAAATATGCCCTCTCCATCGGC	ATGGTCTAGAAAGCTTTACTTGGCAAAATAACTGGGGTTG	pOPINF	*E. coli*
		CAACCCTCACTAAAGGGCATGCAGATGAAGAAAACGGTGG	CTCTGGCGAAGAATTGTTAATTCACTTGGCAAAATAACTGGGGTTG	pESC-Leu	*S. cerevisiae*
TRINITY_DN20_c7_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGTGGAGGACAGCGTCGTTAAAC	GACAACCACAACAAGCACCGTCACTTAGCTTGAGATGATTCCATAAAC	3Ω1	*N. benthamiana*
TRINITY_DN13400_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCGGCAGCAAACCTC	GACAACCACAACAAGCACCGTTAATTCTTGAAGAGGTCTAAGCC	3Ω1	*N. benthamiana*
		AAGTTCTGTTTCAGGGCCCGATGGCGGCAGCAAACCTC	ATGGTCTAGAAAGCTTTATTAATTCTTGAAGAGGTCTAAGCC	pOPINF	*E. coli*
TRINITY_DN3886_c0_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAATAGCCCTAGGGATTGG	GACAACCACAACAAGCACCGTTACTTCTCCAAATTTGAGGATCCTTC	3Ω1	*N. benthamiana*
TRINITY_DN20430_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTATGTCTACTCCTATTCCTTCTCC	GACAACCACAACAAGCACCGTCAATGCTCTTTCTTTTGCAGCA	3Ω1	*N. benthamiana*
TRINITY_DN14690_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTGCCACGCCTCCACC	GACAACCACAACAAGCACCGCTAATTCTGAATATGGACCTGTTTG	3Ω1	*N. benthamiana*
TRINITY_DN52892_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAACTGCTTGATGCAAGGTTG	GACAACCACAACAAGCACCGCTAATCATCACCATTAACAGTAGCAG	3Ω1	*N. benthamiana*
TRINITY_DN5839_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATCATCATGAGTCCGATGAG	GACAACCACAACAAGCACCGTCATTTAAGAAATTCCATGATCCAGTATC	3Ω1	*N. benthamiana*
		AAGTTCTGTTTCAGGGCCCGATGGATCATCATGAGTCCGATG	ATGGTCTAGAAAGCTTTATCATTTAAGAAATTCCATGATCCAG	pOPINF	*E.coli*
TRINITY_DN21159_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATGCAACAAAAGCTGAGG	GACAACCACAACAAGCACCGTCAAGGATAGGCCTCAATGAC	3Ω1	*N. benthamiana*
TRINITY_DN6106_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGGCTCGACCAACAAGAAC	GACAACCACAACAAGCACCGTCATTTATATAACTCGATGATCCAAC	3Ω1	*N. benthamiana*
TRINITY_DN238_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAAAGAGAAGTTCTTCGGCAC	GACAACCACAACAAGCACCGTCAGGTGAGACGCTTGCAGAG	3Ω1	*N. benthamiana*
TRINITY_DN16034_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGTTCAAGGGGAACAAGAAC	GACAACCACAACAAGCACCGTCACAAACGAGAGATCAATAGAGG	3Ω1	*N. benthamiana*
TRINITY_DN15617_c1_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTTCTCTTCATGCTCCTC	GACAACCACAACAAGCACCGTTAACTGGATTTCTGCGGCGG	3Ω1	*N. benthamiana*
TRINITY_DN1230_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTTCTCTTGGATGTCCTTC	GACAACCACAACAAGCACCGCTAAGATGCTGCCCCTGATCTG	3Ω1	*N. benthamiana*

TRINITY_DN15617_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCATCCCTTCCTAATCTCC	GACAACCACAACAAGCACCGTCAGGAAGACGTGGCGGC	3Ω1	*N. benthamiana*
TRINITY_DN89858_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCATCTACTCTCCTTCC	GACAACCACAACAAGCACCGTCAGACATCATCATACACAATTTTGATAC	3Ω1	*N. benthamiana*
TRINITY_DN4762_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTTCTTCCATCCCCTCC	GACAACCACAACAAGCACCGTTAGTCATCAAGCTCAATCTTGATGC	3Ω1	*N. benthamiana*
TRINITY_DN15617_c1_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTTCTCTTCATGCTCCTC	GACAACCACAACAAGCACCGTTAACTGGATTTCTGCGGCG	3Ω1	*N. benthamiana*
TRINITY_DN15617_c1_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTTCTCATCATCATGTTCC	GACAACCACAACAAGCACCGTTACCTGGATGGCGGCGG	3Ω1	*N. benthamiana*
TRINITY_DN12379_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGTTGGGCCACGTCC	GACAACCACAACAAGCACCGTCAACATCCAACGGCGCC	3Ω1	*N. benthamiana*
TRINITY_DN9860_c0_g1_i10	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATCCAGAAACCCCACAG	GACAACCACAACAAGCACCGTTAGCATGTTGCAGTTGATCCA	3Ω1	*N. benthamiana*
TRINITY_DN16027_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAAGCCGCATGCTGTCC	GACAACCACAACAAGCACCGCTAATTTTTAAGGAGAATCTCGTTG	3Ω1	*N. benthamiana*
TRINITY_DN1399_c1_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGGGAGCGGTGGTGG	GACAACCACAACAAGCACCGCTAGTCTTGTTCATCATATCTTAC	3Ω1	*N. benthamiana*
TRINITY_DN334_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCCGCCGCCGCTCT	GACAACCACAACAAGCACCGTTAATTCCTCGGTGCATTGGC	3Ω1	*N. benthamiana*
TRINITY_DN7511_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTGCCGCTCACCC	GACAACCACAACAAGCACCGCTATCTCCTAATATGGGCAACAA	3Ω1	*N. benthamiana*
TRINITY_DN3039_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCTGAGAAGTCCTCCC	GACAACCACAACAAGCACCGTTATATTATGGTCATGGATTGGATG	3Ω1	*N. benthamiana*
TRINITY_DN2103_c0_g2_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGTCACAAGTGACCG	GACAACCACAACAAGCACCGTTATCTAGTGATATGTTTTATGAATGAATC	3Ω1	*N. benthamiana*
TRINITY_DN1286_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAAGGTCCCGTAGATCTG	GACAACCACAACAAGCACCGCTAGGAACAACCAAAGCAAGTC	3Ω1	*N. benthamiana*
TRINITY_DN11457_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGAATCCGAAACTTGACAGAC	GACAACCACAACAAGCACCGTCATTTGATACAGACACCATGC	3Ω1	*N. benthamiana*
TRINITY_DN334_c0_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCCGCCGCCGC	GACAACCACAACAAGCACCGTTAATTCCTCGGTGCATTGG	3Ω1	*N. benthamiana*
TRINITY_DN5184_c0_g1_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGATCCAGAAACCCCAC	GACAACCACAACAAGCACCGTTAAGTTGCAGTTGATCCATATTG	3Ω1	*N. benthamiana*
TRINITY_DN212_c0_g2_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAAACTTTCACTGAGGATG	GACAACCACAACAAGCACCGCTACTCCTCAGCAAGTGGAAC	3Ω1	*N. benthamiana*
TRINITY_DN11987_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGGAGAAGATGAATTGTG	GACAACCACAACAAGCACCGCTAGTTGGACGAACACCAC	3Ω1	*N. benthamiana*
TRINITY_DN12379_c0_g1_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGTTGGGCCACGTC	GACAACCACAACAAGCACCGTCAACATCCAACGGCGCCAC	3Ω1	*N. benthamiana*
TRINITY_DN4525_c0_g2_i2	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCAGGGCAAGAGGCG	GACAACCACAACAAGCACCGTCACGCACTCTTGATGGTCTTC	3Ω1	*N. benthamiana*
TRINITY_DN40246_c0_g2_i1	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGGGGATATGCCACGG	GACAACCACAACAAGCACCGTCAGGTTGCCAAGACACAC	3Ω1	*N. benthamiana*
TRINITY_DN41_c0_g1_i3	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGCGAAGCAGAGAAAATTC	GACAACCACAACAAGCACCGTCAGTACTTCCAGTCATTGGC	3Ω1	*N. benthamiana*

TRINITY_DN41_c0_g1_i4	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCGATGGAGAAGCAGAGGAAG	GACAACCACAACAAGCACCGTCATGCCTTCGTTTTCTCCTTC	3Ω1	*N. benthamiana*
Contig_10649	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGAGTTCATTTTCAAAAGCAGAAAGTAG	GACAACCACAACAAGCACCGTTATGGCAAAAGCTCCTTGATC	3Ω1	*N. benthamiana*
		AAGTTCTGTTTCAGGGCCCGATGAGTTCATTTTCAAAAGCAGAAAG	ATGGTCTAGAAAGCTTTATTATGGCAAAAGCTCCTTGATC	pOPINF	*E. coli*
Contig_6177	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGGTGGTGCAAGTGAAAGTG	GACAACCACAACAAGCACCGTTAATTCGTTTCATCGAAATTTGCC	3Ω1	*N. benthamiana*
Contig_3786	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGAAGAAGAAGAGGGGAG	GACAACCACAACAAGCACCGTCATTTTCTAAGATAATCAATGAATCTC	3Ω1	*N. benthamiana*
Contig_15618	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGTCTCAAAACCACCCAATCAC	GACAACCACAACAAGCACCGCTAAGTGAATGAAGAAATATCTCTGAG	3Ω1	*N. benthamiana*
Contig_20398	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCACCCAAAACCCTACAC	GACAACCACAACAAGCACCGTTAGCATCTTTGATGATTTTTTAGAAAATC	3Ω1	*N. benthamiana*
Contig_9495	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGCCCATCCCTCGAGTCG	GACAACCACAACAAGCACCGTTAAGGTAGAAGAAGGGTGTGC	3Ω1	*N. benthamiana*
Contig_3120	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGTTACTCTTGCACTTTCATGG	GACAACCACAACAAGCACCGTCAATCGGAATCATAGATGATCTTG	3Ω1	*N. benthamiana*
Contig_1707	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCTTCTCCCTTTATCTCAC	GACAACCACAACAAGCACCGTTAATCATCAAGCACAATCTTGACG	3Ω1	*N. benthamiana*
Contig_1193	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCTTCTCTTCCCCTTTGTTC	GACAACCACAACAAGCACCGTTAATTTTCCGCCGCGTAATCAAC	3Ω1	*N. benthamiana*
contig_11686	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCATCTCTTCCACTTCTCTG	GACAACCACAACAAGCACCGTTATGCTTTTTTGTTGGGTGGGTTC	3Ω1	*N. benthamiana*
Contig_14578	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCTTCCCTTCAGTCTTCATG	GACAACCACAACAAGCACCGTCAGGGTGGCGCGGGAATG	3Ω1	*N. benthamiana*
Contig_22550	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGCTTCCCTTCAATCTTCATGC	GACAACCACAACAAGCACCGTCACGCCGATTTGGGCGGC	3Ω1	*N. benthamiana*
Contig_5273	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGACATGCAACTCCTCACC	GACAACCACAACAAGCACCGTCAAGTTGGGAACTTAAGATTCATTGG	3Ω1	*N. benthamiana*
Contig_32188	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGATATATTCCACCAAATACC	GACAACCACAACAAGCACCGTCAATTAGAAGGATTGTGTATAGTAG	3Ω1	*N. benthamiana*
Contig_28595	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGAGAGCTTGAGCACCC	GACAACCACAACAAGCACCGTCAGTTGGAAGAAGGATTGTATAGT	3Ω1	*N. benthamiana*
Contig_33465	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGAATTATCTGCACTTTACTTGC	GACAACCACAACAAGCACCGTTACAAGCTTGTTGATTTCTCTATG	3Ω1	*N. benthamiana*
Contig_31519	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGTACCAGAAAGCCTACGACAAAC	GACAACCACAACAAGCACCGCTAGAGTTTGTTGACGATGAGAGG	3Ω1	*N. benthamiana*
Contig_31446	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGAAAATGGCAAACCAAATACTCAAG	GACAACCACAACAAGCACCGTTAATTAGGTAGGTGATAGTGATAGG	3Ω1	*N. benthamiana*
Contig_14367	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGAGGTGATAGACTTCACCAC	GACAACCACAACAAGCACCGTCAAAGAGGCTGCTTATATGGTG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCATGGAGGTGATAGACTTCACCAC	CTCTGGCGAAGAATTGTTAATTCAAAGAGGCTGCTTATATGGTG	pESC-Leu	*S. cerevisiae*
Contig_30050	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCGATGGAGATTTCATGGTTACTATTAAC	GACAACCACAACAAGCACCGTCAATAAAGGTGGGGCGG	3Ω1	*N. benthamiana*

Contig_11095	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGTTAATGGAGAAAAGAG TAGCC	GACAACCACAACAAGCAC CGCTATAGTCCAACATAG TCAGCC	3Ω1	*N. benthamiana*
Contig_18238	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAGAGTGGCCA TTG	GACAACCACAACAAGCAC CGCTAGGAGTAGCCTATT ACTGC	3Ω1	*N. benthamiana*
Contig_712	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAGAGTAGCCA TTATTGG	GACAACCACAACAAGCAC CGTCAGTTGCCATGCATT CCAAC	3Ω1	*N. benthamiana*
Contig_13076	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAGATCATTTCTTGGTT TCTATTG	GACAACCACAACAAGCAC CGTTAATAAAGGTGGGGT GGAAGTG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCA TGGAGATCATTTCTTGGTTT CTATTG	CTCTGGCGAAGAATTGTT AATTTAATAAAGGTGGGG TGGAAGTG	pESC-Leu	*S. cerevisiae*
Contig_3786	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAAGAAGAAGAGGGGA G	GACAACCACAACAAGCAC CGTCATTTTCTAAGATAAT CAATGAATCTC	3Ω1	*N. benthamiana*
Contig_15618	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGTCTCAAAACCACCCAAT CAC	GACAACCACAACAAGCAC CGCTAAGTGAATGAAGAA ATATCTCTGAG	3Ω1	*N. benthamiana*
Contig_20398	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGCACCCAAAACCCTAC AC	GACAACCACAACAAGCAC CGTTAGCATCTTTGATGA TTTTTTAGAAAATC	3Ω1	*N. benthamiana*
Contig_9495	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGCCCATCCCTCGAGTCG	GACAACCACAACAAGCAC CGTTAAGGTAGAAGAAGG GTGTGC	3Ω1	*N. benthamiana*
TRINITY_DN1050 _c0_g1_i3	*Acanthus ilicifolius*	TTTATGAATTTTGCAGCTCG ATGGCGAAAAGAGTAGCCA TC	GACAACCACAACAAGCAC CGTCACTTGTTGAAATAA CTCGAC	3Ω1	*N. benthamiana*
TRINITY_DN9057 _c0_g1_i13	*Acanthus ilicifolius*	TTTATGAATTTTGCAGCTCG ATGGAGTGGGTGTTGTGG	GACAACCACAACAAGCAC CGTCAGTATAGGTGAGAT GGG	3Ω1	*N. benthamiana*
TRINITY_DN1412 _c0_g1_i3	*Acanthus ilicifolius*	TTTATGAATTTTGCAGCTCG ATGGATCTGTTCACCGTCG	GACAACCACAACAAGCAC CGCTAATCATCATTACTTG ATTCTTG	3Ω1	*N. benthamiana*
Cluster-9204.7837	*Lamium maculatum*	TTTATGAATTTTGCAGCTCG ATGGAGATCATTTCATGGTT AC	GACAACCACAACAAGCAC CGTCAGTAAAGGTGGGGT GGAAG	3Ω1	*N. benthamiana*
Contig_33225	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAGAGTAGCCA TTATTG	GACAACCACAACAAGCAC CGTCAGTTGCCATGCATT CCAAC	3Ω1	*N. benthamiana*
Contig_675	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGAGATTTCATGGTTACT ATTAAC	GACAACCACAACAAGCAC CGTCAATAAAGGTGGGGC GG	3Ω1	*N. benthamiana*
Cluster-2496.6023	*Lamium album*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAGAATAGCCA TTATTGG	GACAACCACAACAAGCAC CGTTAGCTAGTTCCCATT CCAACATAG	3Ω1	*N. benthamiana*
LECA_c33258_g1 _i2	*Leonurus cardiaca*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAACAGAGTAG CCATTATTG	GACAACCACAACAAGCAC CGTTAGGGTAATCCATCA TAGTCTTTG	3Ω1	*N. benthamiana*
PHFR_c68310_g1 _i1	*Phlomis fruticosa*	TTTATGAATTTTGCAGCTCG ATGGAGAAAAGAGTAGCCA TTATTG	GACAACCACAACAAGCAC CGTTAGGTTAATCCATTAT AGTCTGC	3Ω1	*N. benthamiana*
ROOF_c40410_g1 _i1	*Rosmarinus officinalis*	TTTATGAATTTTGCAGCTCG ATGGAGAAACGAGTAGCCA TC	GACAACCACAACAAGCAC CGTCAGACTGCAGAATTG CTGG	3Ω1	*N. benthamiana*
MAVU_c26472_g1 _i2	*Marrubium vulgare*	TTTATGAATTTTGCAGCTCG ATGATGGAGAAAAGAGTGG G	GACAACCACAACAAGCAC CGTCAGATCCCAGGAGC CATTC	3Ω1	*N. benthamiana*
Cluster-2218.4195	*Lamium orvala*	TTTATGAATTTTGCAGCTCG ATGGAGATCATTTCCTGGTT AC	GACAACCACAACAAGCAC CGTCAATAAAGGTGGGGT GAAAGTG	3Ω1	*N. benthamiana*
Cluster-9204.7837	*Lamium maculatum*	TTTATGAATTTTGCAGCTCG ATGGAGATCATTTCATGGTT AC	GACAACCACAACAAGCAC CGTCAGTAAAGGTGGGGT GGAAG	3Ω1	*N. benthamiana*
MAVU_c20842_g1 _i1	*Marrubium vulgare*	TTTATGAATTTTGCAGCTCG ATGGAGAATTCCCTTTCAAT AC	GACAACCACAACAAGCAC CGCTAGTAAAGGTAGTCG GGG	3Ω1	*N. benthamiana*

CYP92A46	*Scoparia dulcis*	TTTATGAATTTTGCAGCTCG ATGGAGAGCTCCTCGGC	GACAACCACAACAAGCAC CGCTAGTAAAGGTGGAGT GGAAG	3Ω1	*N. benthamiana*
Scaffold 2074295	*Wrightia religiosa*	TTTATGAATTTTGCAGCTCG ATGGAAGTTTTAAATAGCAC CAGC	GACAACCACAACAAGCAC CGCTAATAGAGATGGAGA GGTAATC	3Ω1	*N. benthamiana*
LGBX5-like	*Lamium galeobdolon*	TTATGAATTTTGCAGATGGA GGTGATAGACTTCACCAC	GACAACCACAACAAGCAC CGTCAAAGGGAGGGGTT GTATGGTG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCA TGGAGGTGATAGACTTCAC CAC	CTCTGGCGAAGAATTGTT AATTCAAAGGGAGGGGTT GTATGGTG	pESC-Leu	*S. cerevisiae*
LMBx5-like	*Lamium maculatum*	TTTATGAATTTTGCAGCTCG ATGGAGGTGATAAACTTCA CC	GACAACCACAACAAGCAC CGTCAAAGAGGCTGCTCA TATGG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCA TGGAGGTGATAAACTTCAC C	CTCTGGCGAAGAATTGTT AATTCAAAGAGGCTGCTC ATATGG	pESC-Leu	*S. cerevisiae*
LOBx5-like	*Lamium orvala*	TTTATGAATTTTGCAGCTCG ATGGAGGTGATAAACTTCA CC	GACAACCACAACAAGCAC CGTCAAAGAGGCTGCTCA TATGG	3Ω1	*N. benthamiana*
		CAACCCTCACTAAAGGGCA TGGAGGTGATAAACTTCAC C	CTCTGGCGAAGAATTGTT AATTCAAAGAGGCTGCTC ATATGG	pESC-Leu	*S. cerevisiae*
Zm_Bx1	*Zea mays*	TGTTGTTTTTATGAATTTTG CAGATGGCTTTCGCGCCCA AAA	CAGACAACCACAACAAGC TCATGGCAGCGCGTTCTT	3Ω1	*N. benthamiana*
Zm_Bx2	*Zea mays*	TGTTGTTTTTATGAATTTTG CAGATGGCTGCTCAACTGC ATCA	CAGACAACCACAACAAGC TCACGCAGCCTGTGGGA CTA	3Ω1	*N. benthamiana*
Zm_Bx3	*Zea mays*	TGTTGTTTTTATGAATTTTG CAGATGGCCCTTGGAGCTG C	CAGACAACCACAACAAGC TCAGGAAGCAATCCTTGG AACA	3Ω1	*N. benthamiana*
Zm_Bx4	*Zea mays*	TGTTGTTTTTATGAATTTTG CAGATGGCTCTCGAAGCAG CGTA	CAGACAACCACAACAAGC TCATTTGGGAATTCTAGG AACAAGG	3Ω1	*N. benthamiana*
Zm_Bx5	*Zea mays*	TGTTGTTTTTATGAATTTTG CAGATGGCACTCCAGGCAG C	CAGACAACCACAACAAGC CTAGACGGCCCTAGGAAC AAG	3Ω1	*N. benthamiana*
Zm_Bx6	*Zea mays*	TTTATGAATTTTGCAGCTCG ATGGCTCCAACGACCGCCA C	GACAACCACAACAAGCAC CGCTAGAGCCTGAAGTG GTCGAG	3Ω1	*N. benthamiana*
Zm_Bx7	*Zea mays*	TTTATGAATTTTGCAGCTCG ATGGGGCACCAGGCGCAG	GACAACCACAACAAGCAC CGTCACGGGAAGACCTC GATGATG	3Ω1	*N. benthamiana*
Zm_Bx8	*Zea mays*	TTTATGAATTTTGCAGCTCG ATGGCAGCATCGTGCGGC	GACAACCACAACAAGCAC CGTCAGTAGGAGTTTATG AGATGAACC	3Ω1	*N. benthamiana*
EU747715	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGCTTCTTCTCTCAAGG CAAC	GACAACCACAACAAGCAC CGTCAAGAAAGTGCAGAT TTCAAACTTTTG	3Ω1	*N. benthamiana*
EU747716	*Lamium galeobdolon*	TTTATGAATTTTGCAGCTCG ATGGCCGCTAATTCTCTCAA GTC	GACAACCACAACAAGCAC CGTCAAACAAGTGCAGAT TTTAAGGTTTTG	3Ω1	*N. benthamiana*
EU747711	*Aphelandra squarrosa*	TTTATGAATTTTGCAGCTCG ATGGCTGCTGCTGCTCTCA AAG	GACAACCACAACAAGCAC CGCTATAACAGAACTGAA TCACCCCTC	3Ω1	*N. benthamiana*

Supplemental Table 3: Accession numbers (NCBI) for genes characterized in this study.

Gene name	Specie	NCBI Accession
AsFMO	*Aphelandra squarrosa*	OQ921353
AsBx4	*Aphelandra squarrosa*	OQ921354
AsBx5	*Aphelandra squarrosa*	OQ921355
AsBx6	*Aphelandra squarrosa*	OQ921356
AsBx7	*Aphelandra squarrosa*	OQ921357
AsPPO-1	*Aphelandra squarrosa*	OQ921358
AsPPO-2	*Lamium galeobdolon*	OQ921359
LgBx4	*Lamium galeobdolon*	OQ921360
LgFMO	*Lamium galeobdolon*	OQ921361
LgBx5	*Lamium galeobdolon*	OQ921362
LgBx8	*Lamium galeobdolon*	OQ921363
LgBx5-like	*Lamium galeobdolon*	OQ921364
LmBx5-like	*Lamium maculatum*	OQ921365
LoBx5-like	*Lamium orvala*	OQ921366
WrBx4	*Wrightia religiosa*	OQ921367
AiFMO	*Acanthus ilicifolius*	OQ921368
AiBx4	*Acanthus ilicifolius*	OQ921369
AiBx5	*Acanthus ilicifolius*	OQ921370
SiFMO	*Sesamum indicum*	OQ921371
MvFMO	*Marrubium vulgare*	OQ921372
RoFMO	*Rosmarinus officinalis*	OQ921373
PfFMO	*Phlomis fruticosa*	OQ921374
LcFMO	*Leonurus cardiaca*	OQ921375
LaFMO	*Lamium album*	OQ921376
LmFMO	*Lamium maculatum*	OQ921377
LgFMO_ctr33225	*Lamium galeobdolon*	OQ921378

Supplemental data set 1

Sequences used for FMO phylogeny

>Aphelandra squarrosa_FMO
MQMKKTVAIVGAGISGLLACKYALSIGLNPVVFEAESCVGGLWNHTIECTRLQSLKDYFQFSDFPWPSTIKTMFPKNEQVLEYVNSYADHFQLR
QYVRFNSQVMSIGYEGESKEEMGSWKSWGGTGMAFGSKGKWRLQVEAGGGMQEYHVADFVIVCIGKFSGLPAVPDFPPGSGPEVFSGKVL
HSMEYSDMDNASAAQFIKGKRIAVIGSGKSAIDIAFECGRANGRDTPCTVIHRNAHWMLPYEKPWLLAFALLCFTRFSELLVHKPGEGFFASVL
ATLLSPLRWLVSKFLEISLRWRLPLKKYDMIPSENFLEEANSCQVIMLQDNFFDKVVDGSIVFKKSEHFSFCKEGLIVEGEDSPVEVDIVIFATGY
KGDEKLKNIFASPAFQNHIFGSRNSTVPLFRQMIHPRIPQLAVVGYSESLSNLYTFEMRCRWLAFFLDRAFALPSIKEMEDDIKMWERYMKKYA
GNGEFKRGCIAGIHIWYNDQLCKDIGCSPRRKKGISSELFQPYGLEDYDGITPAQINPSYFAK
>Lamium galeobdolon_FMO
MLMEKRVAIIGAGISGLLACKYTASIGLKPIVFEEQDNVGGLWNHTIEITRLQQVKQSFQFSDFPWPSSADDNPKSAQLLEYLQSYAHNFDLLPFI
KFNSKVMNIDYVGESKEEMQSWDLWGGSGKAFGSKGRWILKVLHTKDDSIKVYESEFVVVCTGRFSGLPNIPEFAPGYGPEIFSGQALHSMD
YSNMDNASAAQFIKGKRIAIIGSGKSAFDIAFECANANGNDNPCTLVQRTIHWTFPSTKTWGLNFAFLIFTRFSELMVRKPGQGFVSSALTTLLT
PLRWGMSKYVESYLRWKLPLKKYGMVPKESFAAESSACQIFYLPRGNFYGKVEDGSIVLKKSQEFRFCKHGLIIDGDDKNPINADIVIFATGYK
GDEKLKSIFASPTFQNYIVGSPTSTVPLYRQMIQPRIPQLAVIGYSESFSNLFTFEMRCKWLAFFLDQAFELPSIRAMKKEVEAWETYMKRYAG
NNKFRRACIGRVHVWYNDQLCRDIGCNPRRKKGFLSELFEPYGSADYVGL
>Sesamum indicum_XM_011080249.2
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Sequences used for BX5 phylogenetic analysis

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Sequences for BX6 phylogenetic analysis

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Sequences for BX7 phylogenetic analysis

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Sequences used for BX8 phylogenetic analysis

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Supplemental data set 2

Supplementary NMR data

NMR data for isolated BXDs (HBOA-Glc, DIBOA-Glc, DIMBOA-Glc and TRIBOA-Glc

HBOA-Glc ((2*R*)-2-*O*-β-D-glucopyranosyl-2*H*-1,4-benzoxazin-3(4*H*)-one)

^{1}H-NMR (700 MHz, MeOH-d_3) δ ppm: 7.07 (*m*, 1H, H-6), 7.003 (*m*, 1H, H-5), 6.998 (*m*, 1H, H-8), 6.92 (*m*, 1H, H-7), 5.74 (*s*, 1H, H-2), 4.68 (*d*, *J*= 7.8 Hz, 1H, H-1'), 3.85 (*dd*, *J*= 12.0, 1.5 Hz, 1H, H-6'), 3.69 (*dd*, *J*= 12.0, 4.6 Hz, 1H, H-6'), 3.36 (*dd*, *J*= 8.5, 8.4 Hz, 1H, H-3'), 3.314 (*m*, 1H, H-5'), 3.31 (*m*, 1H, H-4'), 3.19 (*dd*, *J*= 8.4, 7.8 Hz, 1H, H-2'). ^{13}C-NMR (175 MHz, MeOH-d_3) δ ppm: 163.2 (C-3), 142.0 (C-9), 127.1 (C-10), 125.0 (C-5), 124.1 (C-8), 118.9 (C-6), 116.7 (C-7), 103.9 (C-1'), 96.4 (C-2), 78.3 (C-5'), 77.9 (C-3'), 71.9 (C-2'), 71.1 (C-4'), 62.6 (C-6'). The chemical shifts were in agreement with published data (Yin et al., 2008). HRMS (ESI-TOF, negative) *m/z*: calc'd for $C_{14}H_{16}NO_8$ $[M-H]^-$ 326.0875, found 326.0866 .

DIBOA-Glc ((2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one)

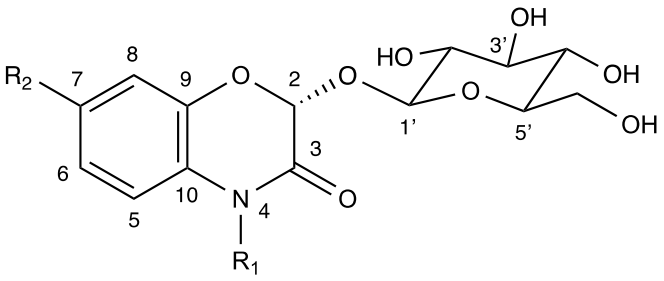
^{1}H-NMR (700 MHz, MeOH-d_3) δ ppm: 7.36 (*m*, 1H, H-5), 7.07-7.13 (*m*, 3H, H-6, 7, 8), 5.93 (*s*, 1H, H-2), 4.67 (*d*, *J*= 8.0 Hz, 1H, H-1'), 3.85 (*bd*, *J*= 11.8 Hz, 1H, H-6'), 3.68 (*dd*, *J*= 11.8, 4.5 Hz, 1H, H-6'), 3.28-3.36 (*m*, 3H, H-3', H-4'. H-5'), 3.18 (*dd*, *J*= 8.4, 8.0 Hz, 1H, H-2'). ^{13}C-NMR (175 MHz, MeOH-d_3) δ ppm: 158.1 (C-3), 142.2 (C-9), 129.1 (C-10), 125.7 (C-6), 124.1 (C-7), 118.6 (C-8), 114.2 (C-5), 103.6 (C-1'), 97.7 (C-2), 78.4 (C-3'), 77.9 (C-5'), 74.8 (C-2'), 71.0 (C-4'), 62.5 (C-6'). The chemical shifts were in agreement with published data (Yin et al., 2008). HRMS (ESI-TOF, negative) *m/z*: calc'd for $C_{14}H_{16}NO_9$ $[M-H]^-$ 342.0825, found 342.0825. $[\alpha]_D^{20}$ +66.1 (c 0.47, H_2O).

TRIBOA-Glc ((2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-7-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one)

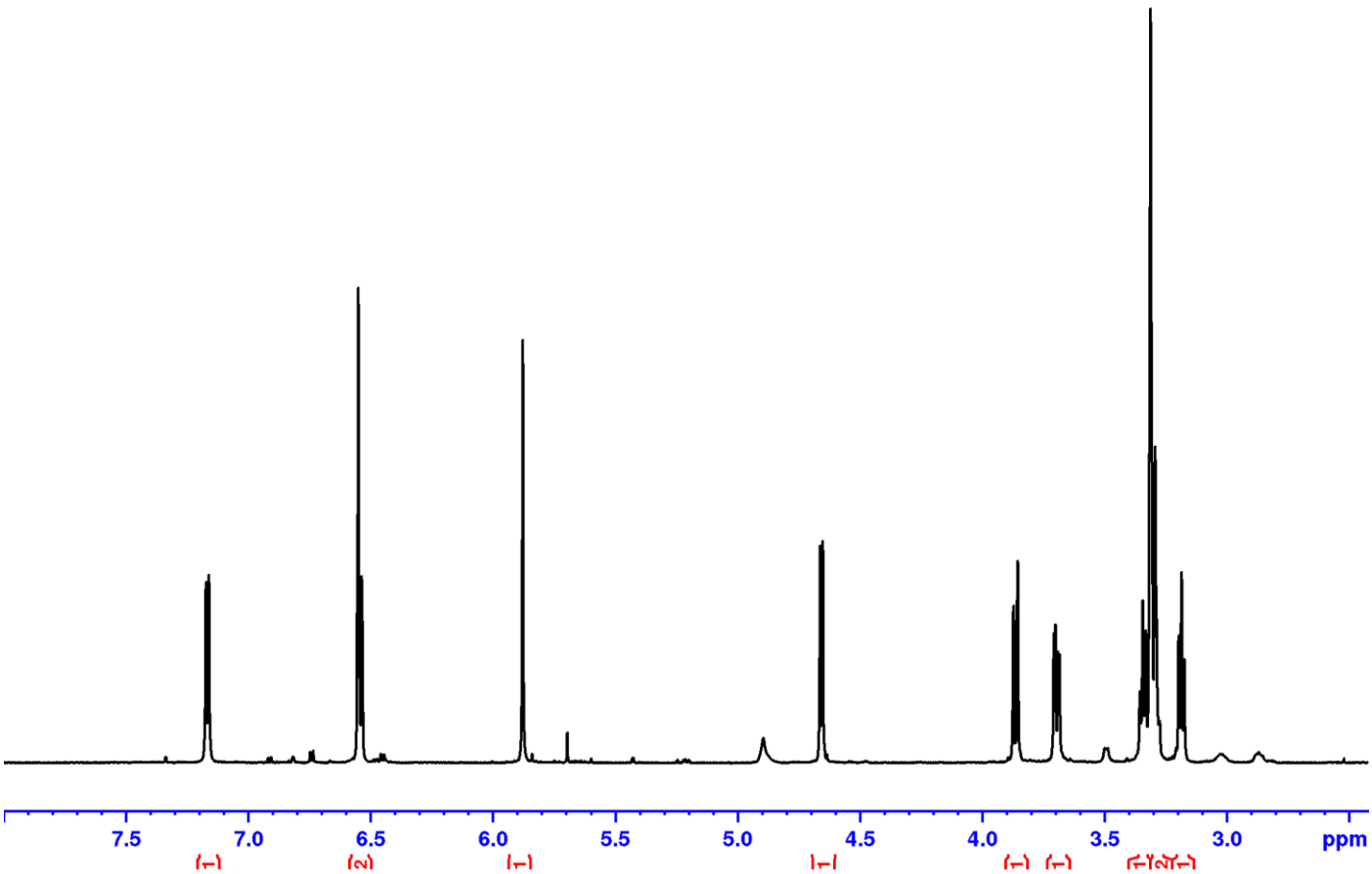
^{1}H-NMR (700 MHz, MeOH-d_3) δ ppm: 7.17 (*d*, *J*= 8.4 Hz, 1H, H-5), 6.55 (*d*, *J*= 2.6 Hz, 1H, H-8), 6.54 (*dd*, *J*= 8.4, 2.6 Hz, 1H, H-8), 5.88 (*s*, 1H, H-2), 4.66 (*d*, *J*= 7.9 Hz, 1H, H-1'), 3.86 (*dd*, *J*= 12.1, 1.4 Hz, 1H, H-6'), 3.69 (*dd*, *J*= 12.1, 4.7 Hz, 1H, H-6'), 3.34 (*dd*, *J*= 8.6, 8.4 Hz, 1H, H-3'), 3.294 (*m*, 1H, H-4'), 3.289 (*m*, 1H, H-5'), 3.18 (*dd*, *J*= 8.4, 7.9 Hz, 1H, H-2'). ^{13}C-NMR (175 MHz, MeOH-d_3) δ ppm: 157.2 (C-3), 156.3 (C-7), 143.3 (C-9), 121.7 (C-10), 115.0 (C-5), 110.7 (C-6), 105.8 (C-8), 103.6 (C-1'), 98.0 (C-2), 78.4 (C-5'), 77.9 (C-3'), 74.8 (C-2'), 71.0 (C-4'), 62.5 (C-6'). HRMS (ESI-TOF, positive) *m/z*: calc'd for $C_{14}H_{18}NO_{10}$ $[M+H]^+$ 360.0925, found 360.0919.

DIMBOA-Glc ((2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one)

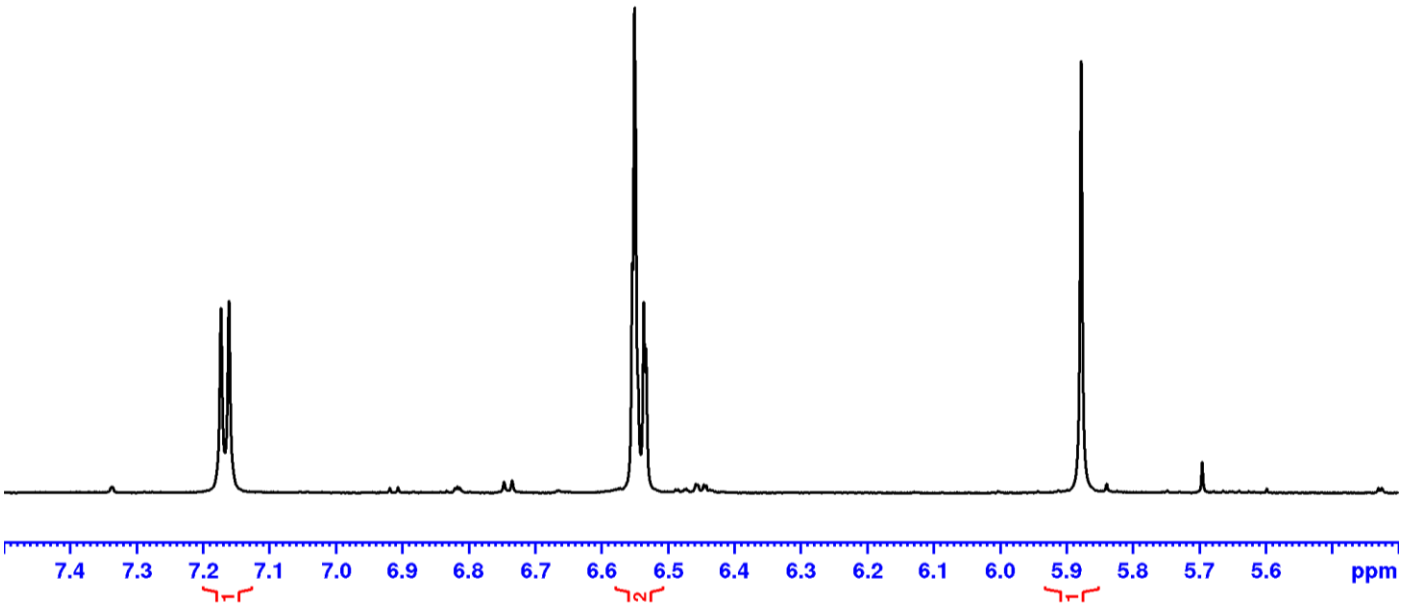
^{1}H-NMR (700 MHz, Acetone-d_6) δ ppm: 7.27 (*d*, *J*= 8.9 Hz, 1H, H-5), 6.76 (*d*, *J*= 2.6 Hz, 1H, H-8), 6.70 (*dd*, *J*= 8.9, 2.6 Hz, 1H, H-6), 5.85 (*s*, 1H, H-2), 4.71 (*d*, *J*= 8.0 Hz, 1H, H-1'), 3.88 (*dd*, *J*= 11.7, 2.6 Hz, 1H, H-6'), 3.78 (*s*, 3H, O$\underline{CH_3}$), 3.65 (*dd*, *J*= 11.7, 5.8 Hz, 1H, H-6'), 3.40 (*dd*, *J*= 9.0, 9.0 Hz, 1H, H-3'), 3.38 (*m*, 1H, H-5'), 3.30 (*dd*, *J*= 9.2, 9.0 Hz, 1H, H-4'), 3.17 (*dd*, *J*= 9.0, 8.0 Hz, 1H, H-2'). ^{13}C-NMR (175 MHz, Acetone-d_6) δ ppm: 156.9 (C-7), 154.8 (C-3), 141.9 (C-9), 122.0 (C-10), 113.6 (C-5), 108.5 (C-6), 103.6 (C-8), 97.2 (C-2), 103.0 (C-1'), 77.3 (C-5'), 77.0 (C-3'), 73.8 (C-2'), 70.4 (C-4'), 62.0 (C-6'). The chemical shifts were in agreement with published data (Wouters et al., 2014). HRMS (ESI-TOF, positive) *m/z*: calc'd for $C_{15}H_{20}NO_{10}$ $[M+H]^+$ 372.0930, found 372.0924.



R_1 = OH, R_2 = H	DIBOA-Glc
R_1 = H, R_2 = H	HBOA-Glc
R_1 = OH, R_2 = OCH_3	DIMBOA-Glc
R_1 = OH, R_2 = OH	TRIBOA-Glc



TRIBOA-Glc. ^{1}H NMR with water suppression, full range in MeOH-d_3



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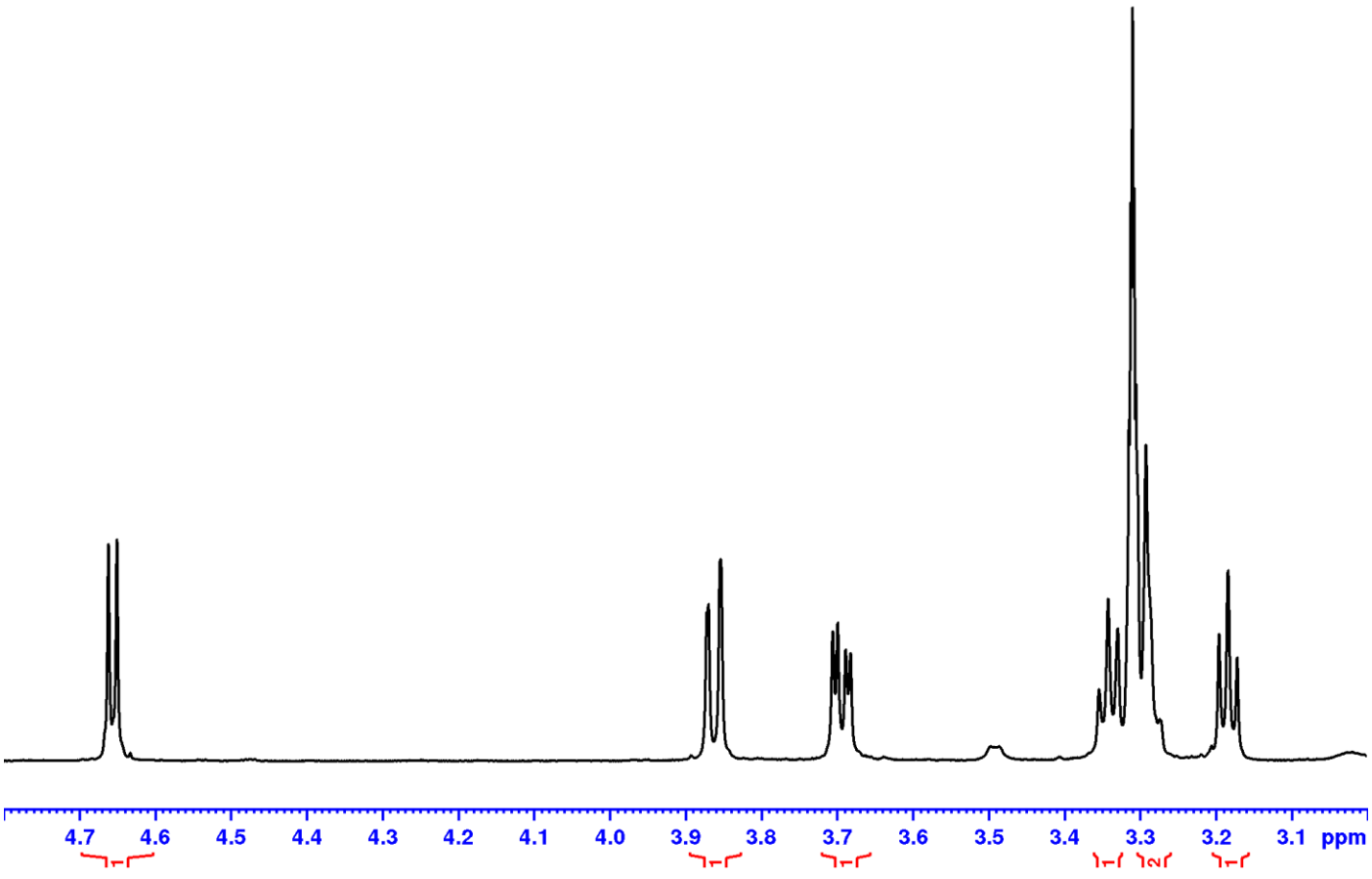
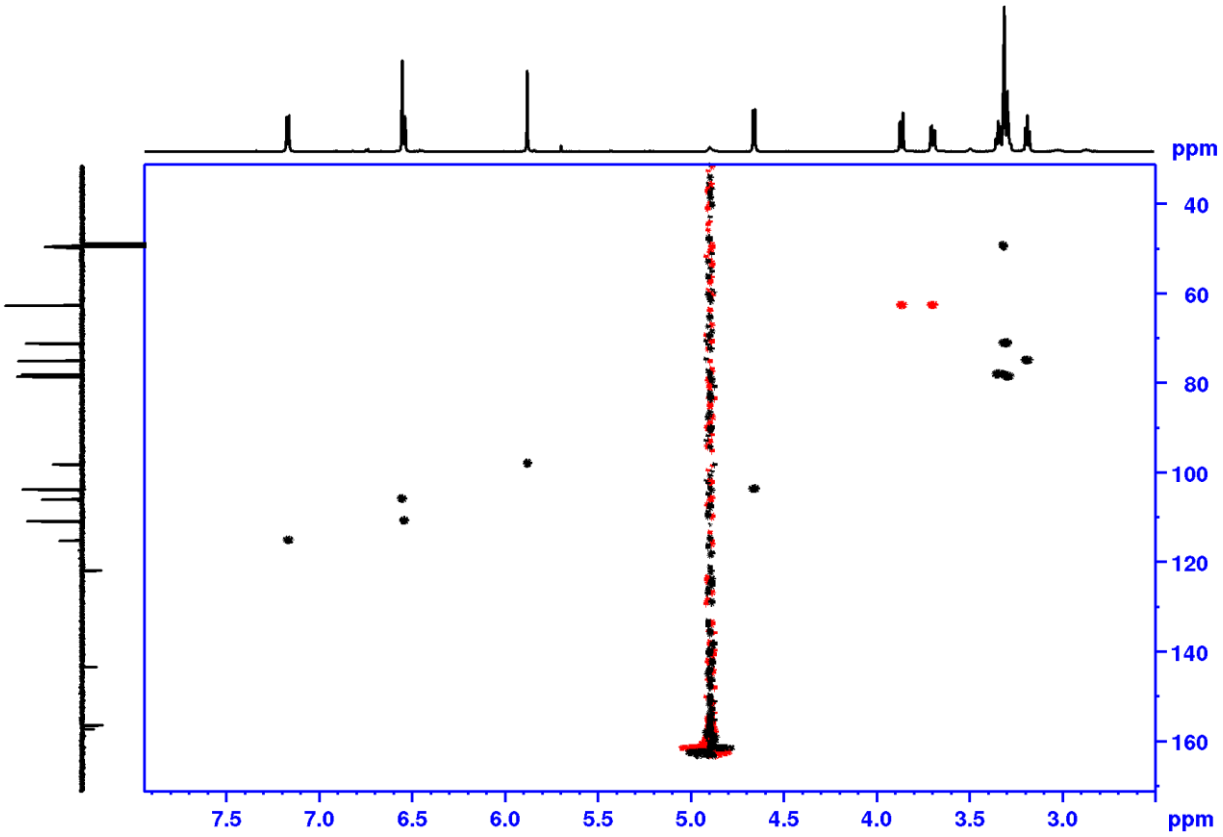
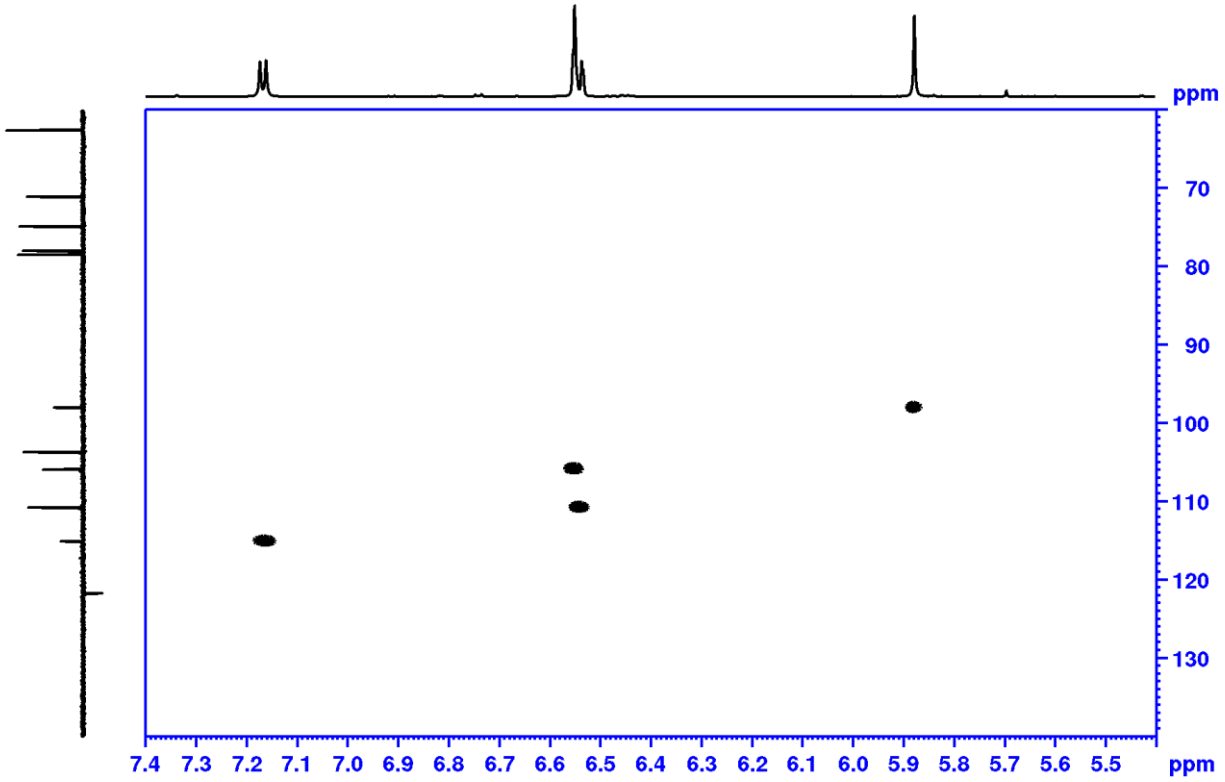


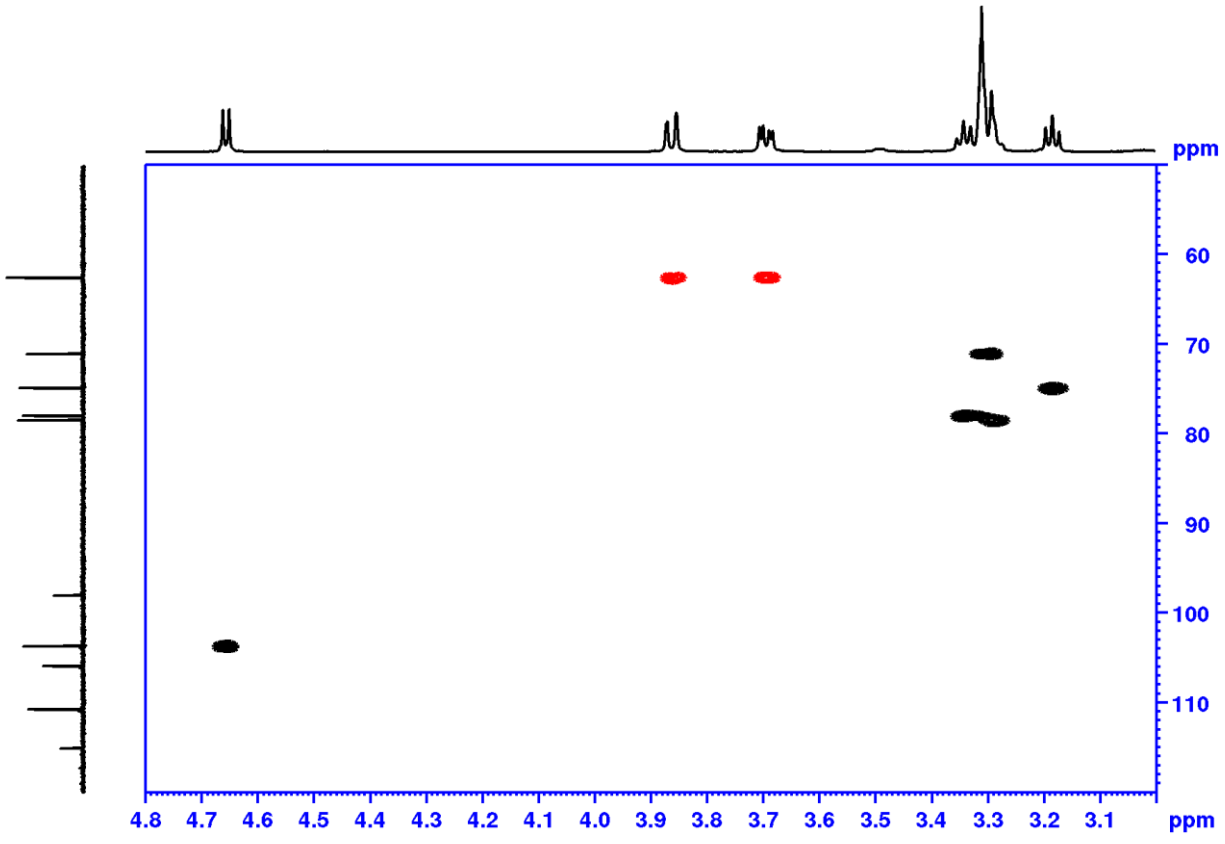
TRIBOA-Glc. ^{1}H NMR with water suppression, aliphatic range in MeOH-d_3



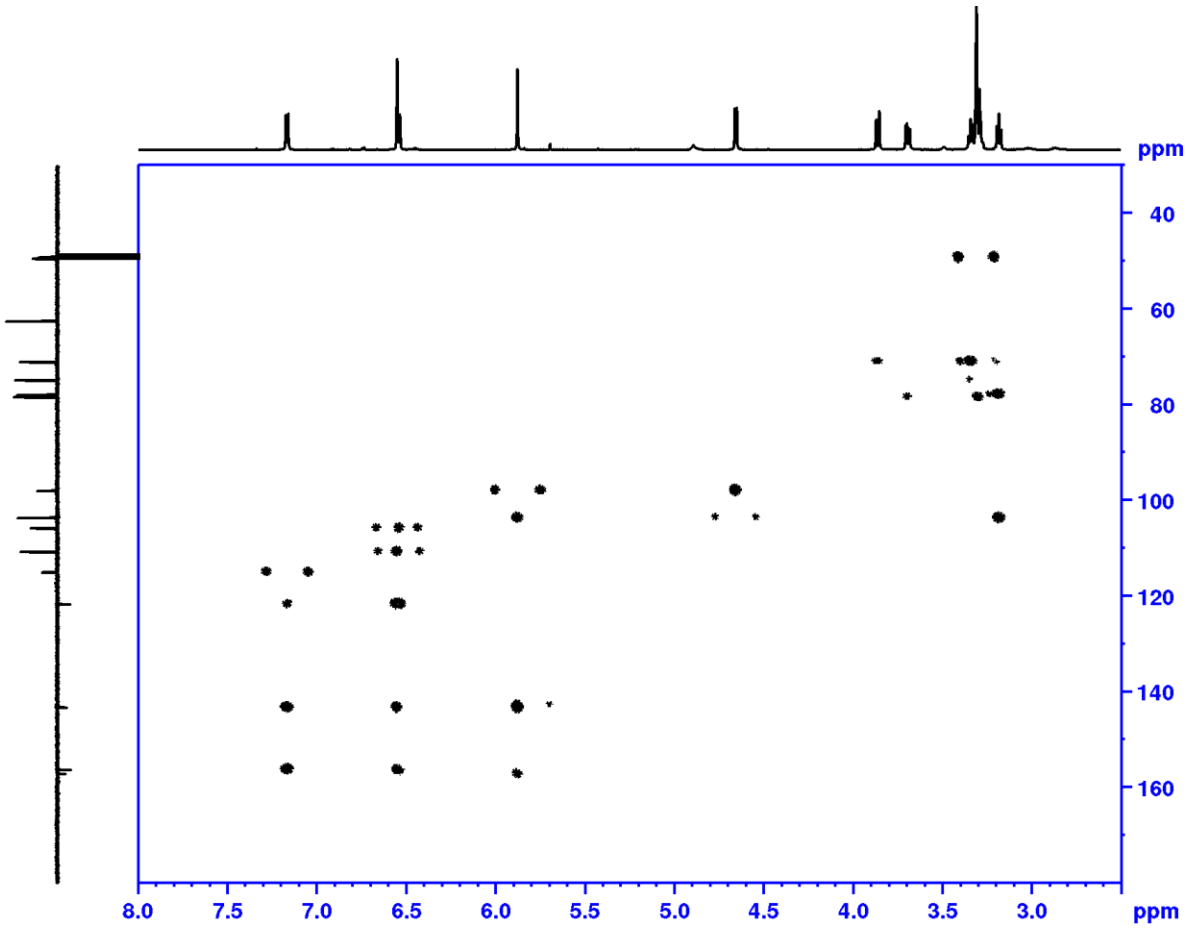
TRIBOA-Glc. phase sensitive HSQC, full range in MeOH-d_3



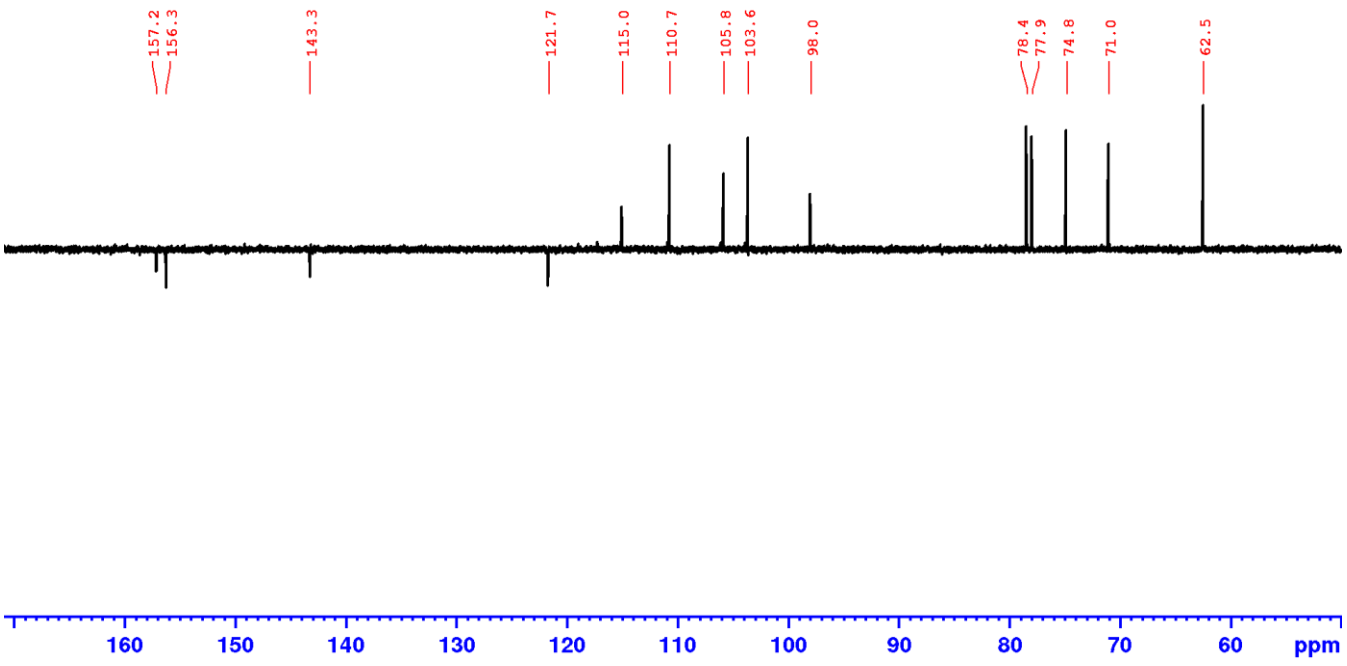
TRIBOA-Glc. phase sensitive HSQC, aromatic range in MeOH-d_3



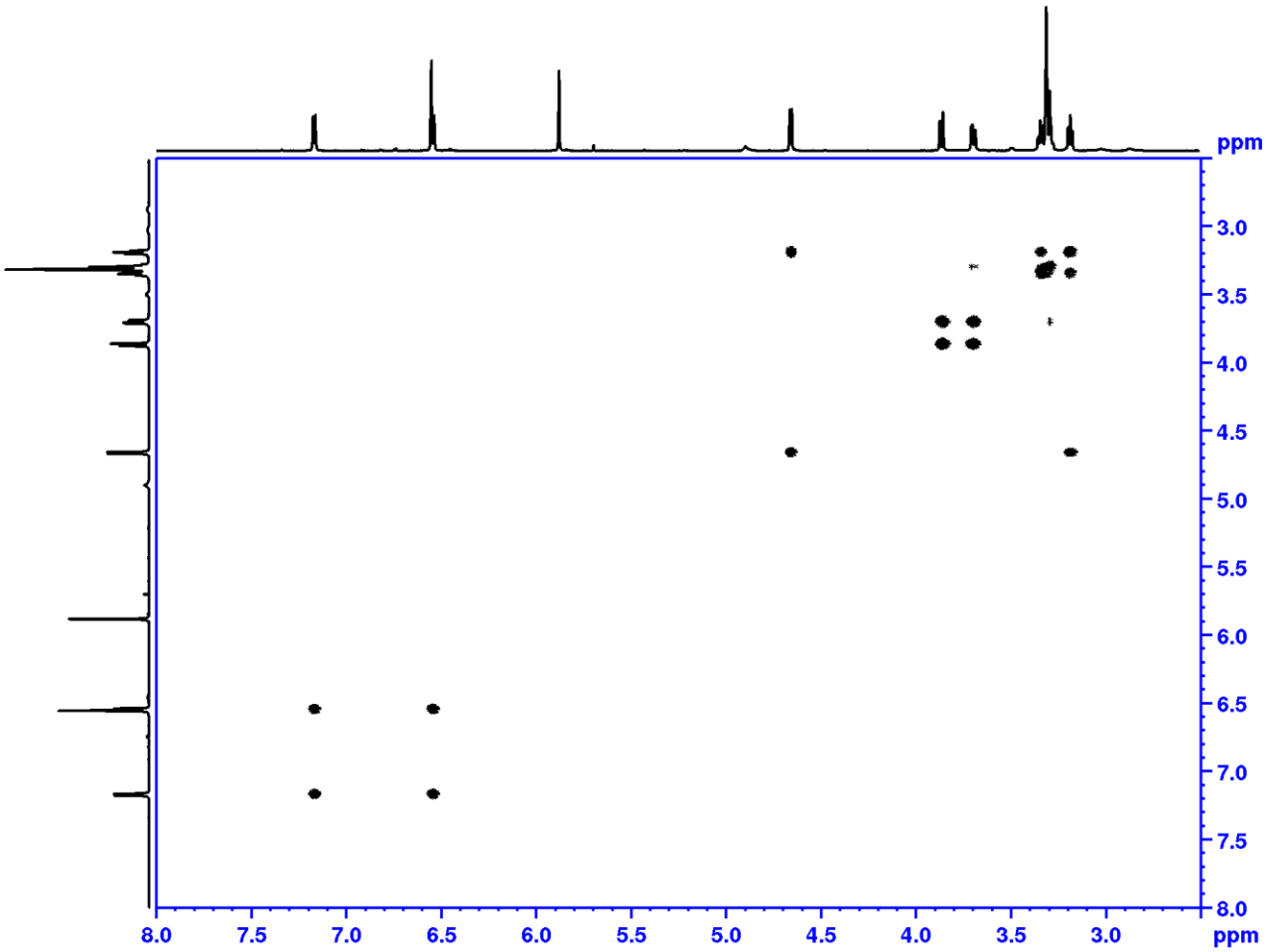
TRIBOA-Glc. phase sensitive HSQC, aliphatic range in MeOH-d_3



TRIBOA-Glc. HMBC, full range in MeOH-d_3



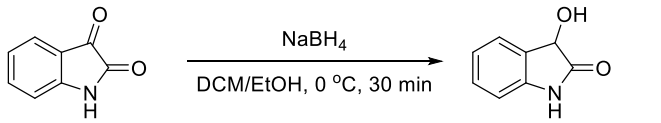
TRIBOA-Glc. ^{13}C (DEPTQ), full range in MeOH-d_3



TRIBOA-Glc. ^{1}H-^{1}H DQF COSY with water suppression, full range in MeOH-d_3

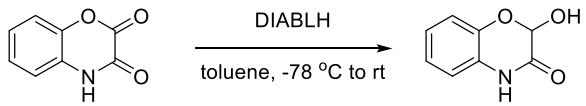
Synthesis and Characterization of compounds

NMR spectra were measured on a 400 MHz Bruker Avance III HD spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). CD3OD and $CDCl_3$ were used as solvents. NMR spectra were referenced to the residual solvent signals at δ_H 3.31 and δ_C 49.0 for CD3OD and δ_H 7.26 and δ_C 77.0 for $CDCl_3$. For spectrometer control and data processing Bruker TopSpin ver. 3.6.1 was used.



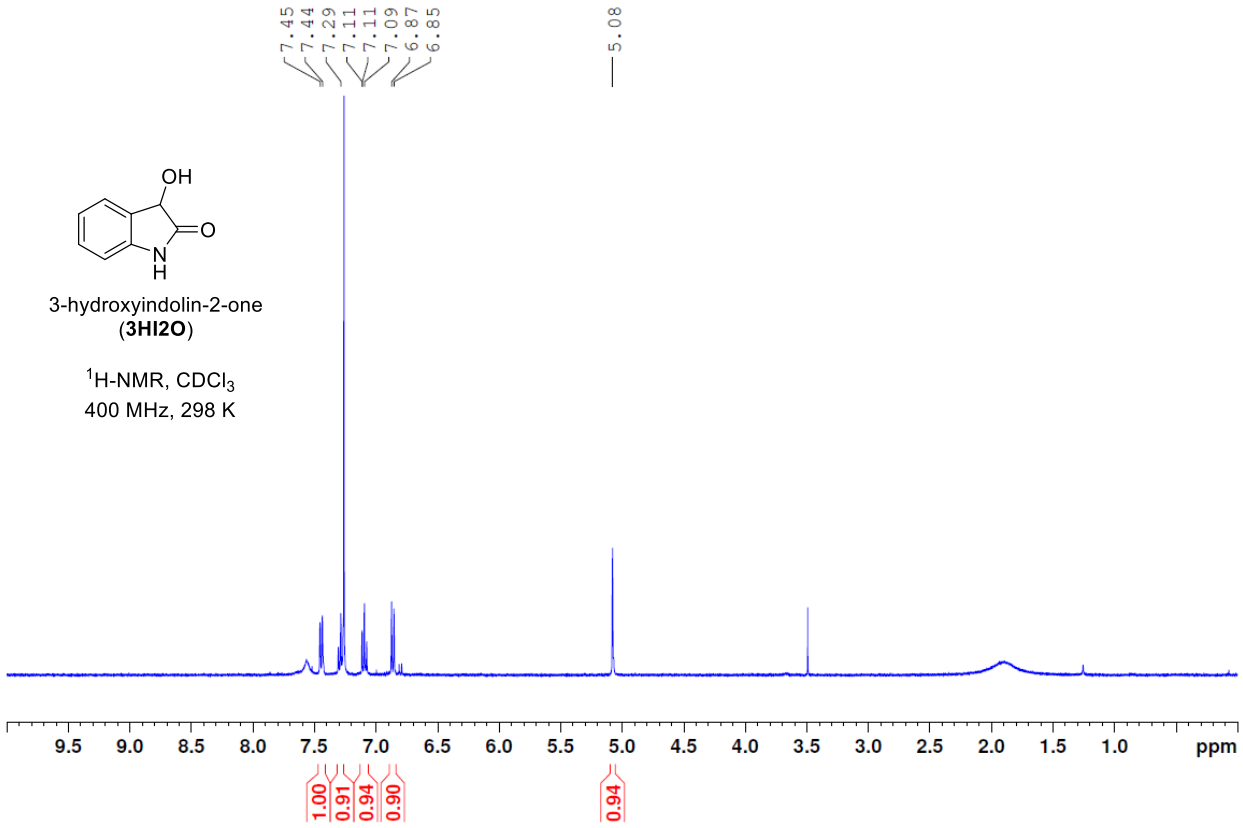
isatin — 3-hydroxyindolin-2-one (**3HI2O**)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 7.4 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 5.08 (s, 1H);
^{13}C-NMR (101 MHz, $CDCl_3$) δ 178.0, 140.8, 129.9, 127.1, 125.6, 123.2, 110.1, 70.0.

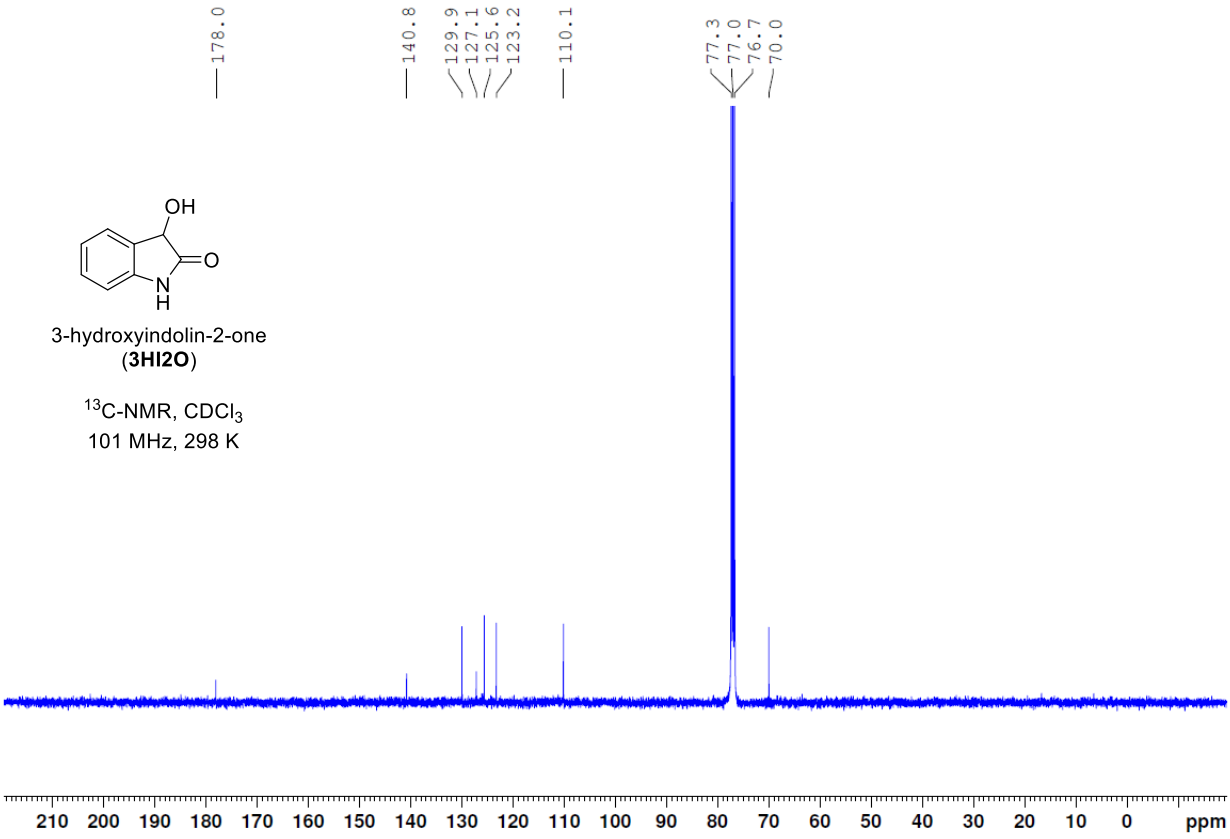


2,3-dioxo-1,4-benzoxazine — **HBOA**

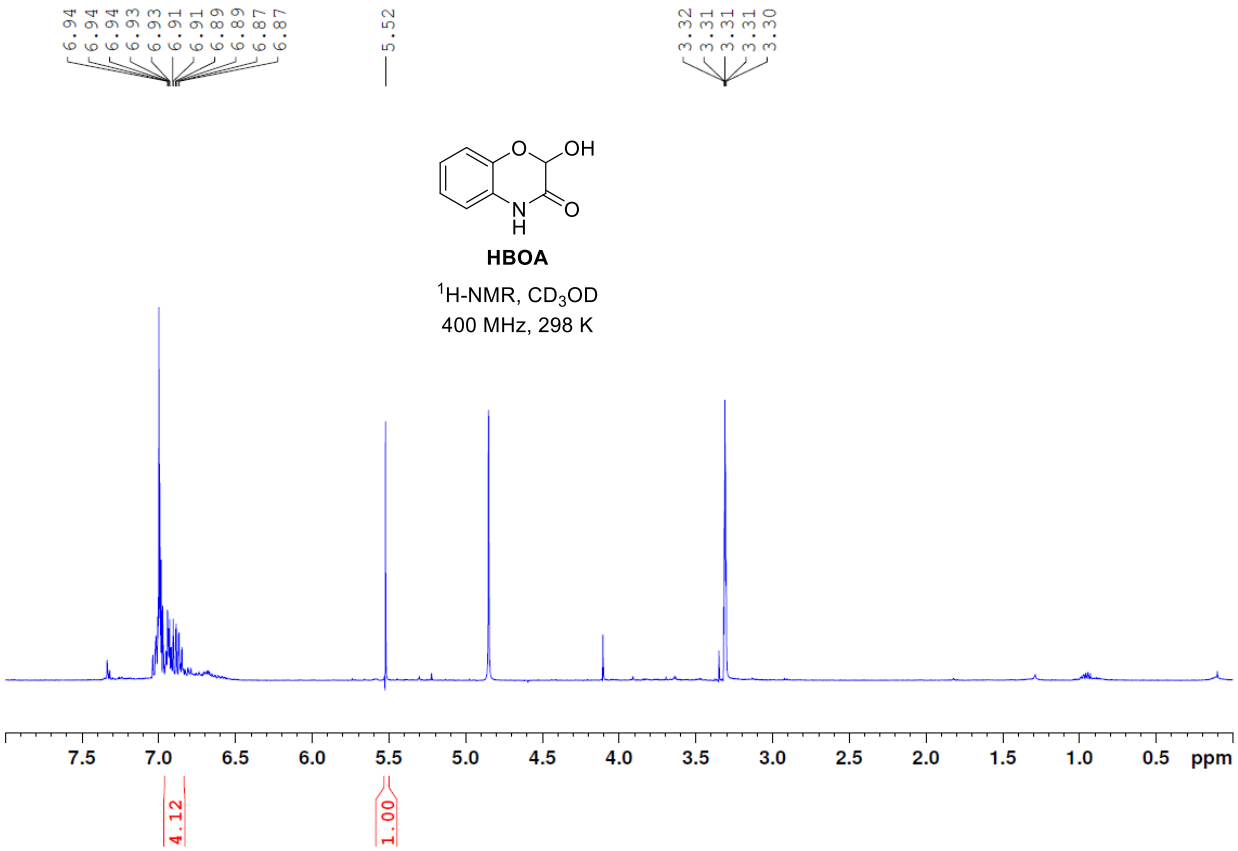
^{1}H-NMR (400 MHz, CD_3OD) δ 6.93 (m, 4H), 5.52 (s, 1H);
^{13}C-NMR (101 MHz, CD_3OD) δ 165.3, 142.4, 127.5, 124.9, 123.7, 118.8, 116.8, 92.1.



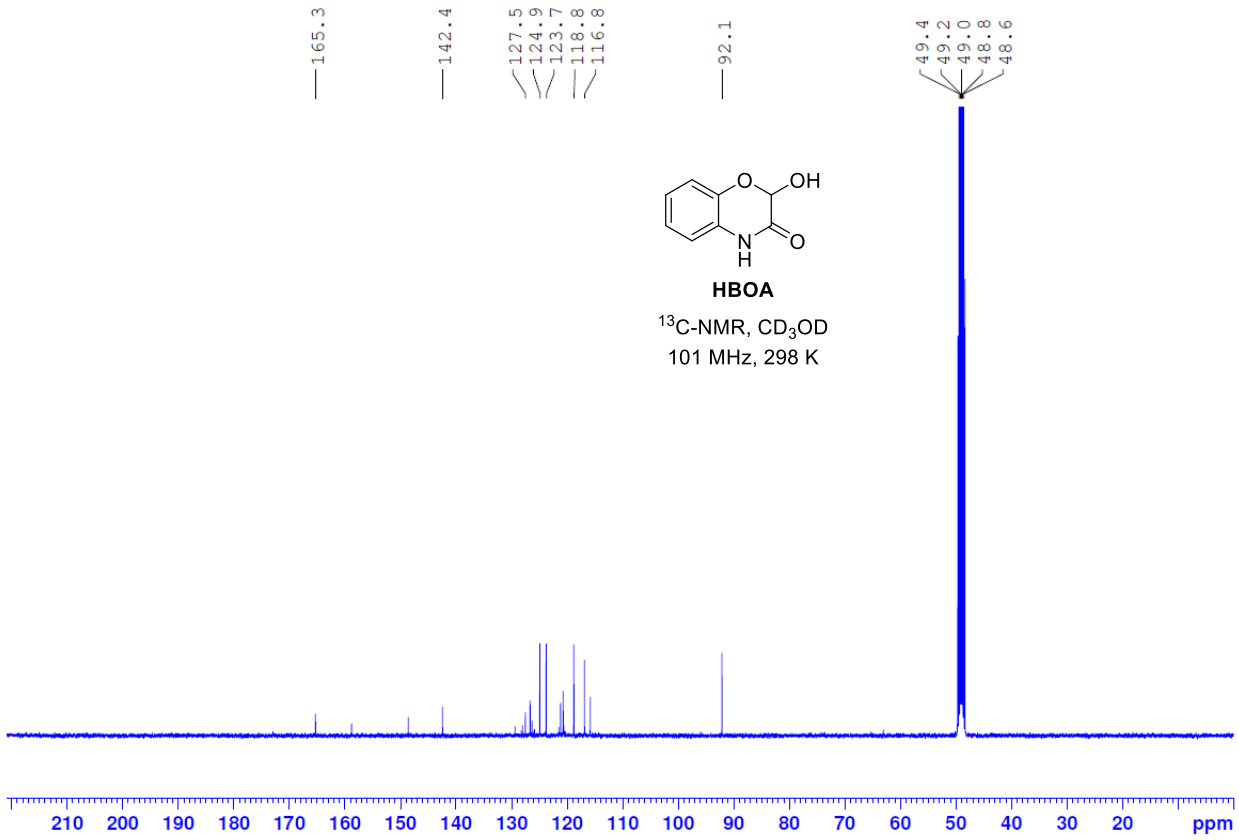
3HI2O, ^{1}H-NMR, CDCl3 400MHz, 298K.



SI Figure 22: **3HI2O**, ^{13}C-NMR, CDCl3 101MHz, 298K.



HBOA, ^{1}H-NMR, CDC3OD 400MHz, 298K.



HBOA, ^{13}C-NMR, CD3OD 101MHz, 298K.

Literature:

Yin H, Zhang S, Luo X, Liu Y (2008) Preparative isolation and purification of two benzoxazinoid glucosides from *Acanthus ilicifolius* L. by high-speed counter-current chromatography. *J Chromatogr A* 1205: 177-181

Wouters FC, Reichelt M, Glauser G, Bauer E, Erb M, Gershenzon J, Vassao GV (2014) Reglucosylation of the Benzoxazinoid DIMBOA with Inversion of Stereochemical Configuration is a Detoxification Strategy in Lepidopteran Herbivores. *Angew Chem Int Ed* 53: 11320-11324