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A Rationally Designed Agonist Defines Subfamily IIIA Abscisic Acid Receptors As Critical Targets for Manipulating Transpiration

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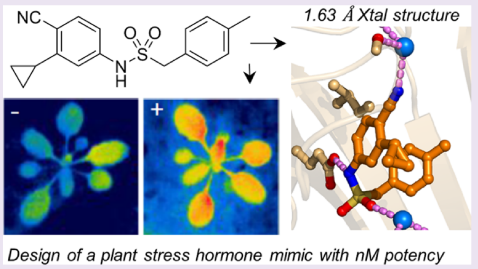
Supporting Information

ABSTRACT: Increasing drought and diminishing freshwater supplies have stimulated interest in developing small molecules that can be used to control transpiration. Receptors for the plant hormone abscisic acid (ABA) have emerged as key targets for this application, because ABA controls the apertures of stomata, which in turn regulate transpiration. Here, we describe the rational design of cyanabactin, an ABA receptor agonist that preferentially activates *Pyrabactin Resistance 1* (PYR1) with low nanomolar potency. A 1.63 Å X-ray crystallographic structure of cyanabactin in complex with PYR1 illustrates that cyanabactin's arylnitrile mimics ABA's cyclohexenone oxygen and engages the tryptophan lock, a key component required to stabilize activated receptors. Further, its sulfonamide and 4-methylbenzyl substructures mimic ABA's carboxylate and C6 methyl groups, respectively. Isothermal titration calorimetry measurements show that cyanabactin's compact structure provides ready access to high ligand efficiency on a relatively simple scaffold. Cyanabactin treatments reduce *Arabidopsis* whole-plant stomatal conductance and activate multiple ABA responses, demonstrating that its *in vitro* potency translates to ABA-like activity *in vivo*. Genetic analyses show that the effects of cyanabactin, and the previously identified agonist quinabactin, can be abolished by the genetic removal of PYR1 and PYL1, which form subclade A within the dimeric subfamily III receptors. Thus, cyanabactin is a potent and selective agonist with a wide spectrum of ABA-like activities that defines subfamily IIIA receptors as key target sites for manipulating transpiration.



Design of a plant stress hormone mimic with nM potency

There is strong interest in developing strategies to mitigate the effects of abiotic stress on agricultural productivity. Among abiotic stresses, drought is the major source of crop losses, and its impact is anticipated to grow as a consequence of climate change. Drought reduces plant growth by limiting water; however its effects on yield vary quantitatively throughout a growing season and are generally highest during flowering when drought can cause reproductive failure. One strategy to mitigate drought effects on yield is to preventively reduce transpiration early in a crop's growing season so that soil water levels are higher during flowering, thus providing a chemical analog of deficit irrigation that can be used in rain-fed environments.[1] New small molecules that enable one to rationally tune transpiration in anticipation of drought will, therefore, be valuable management tools for maximizing water productivity. Receptors for the plant hormone abscisic acid **1** (ABA) have emerged as excellent targets for such applications; upon water deficit, mRNAs for key ABA-biosynthetic genes are transcribed,[2] and the ensuing elevation of ABA triggers guard cell closure, which reduces water loss due to transpiration and induces other protective responses.[3] ABA treatments can increase crop yields during drought,[4,5] but ABA's photoinstability, rapid metabolism, and relatively high cost limit its suitability for widespread agricultural use.[1] Thus, new synthetic scaffolds that mimic ABA action with improved properties are of interest.

ABA executes its effects through a family of soluble receptors that are found throughout land plants.[6] ABA binds directly to PYR/PYL/RCAR (*Pyrabactin Resistance 1/PYR1-Like/Regulatory Component of ABA Receptor*) proteins.[7,8] Upon binding, the ligand−receptor complex is stabilized in a conformation that binds to and inhibits members of the clade A subfamily of type II C protein phosphatases (PP2Cs).[9,10] A key function of these PP2Cs is to dephosphorylate and inactivate downstream stress-activated *SNF1-Related Protein Kinase Subfamily 2* kinases (SnRK 2s),[7,11] which in turn allows SnRK2 autoactivation and subsequent phosphorylation of downstream targets such as transcription factors[12−14] and guard cell anion channels,[15,16] the

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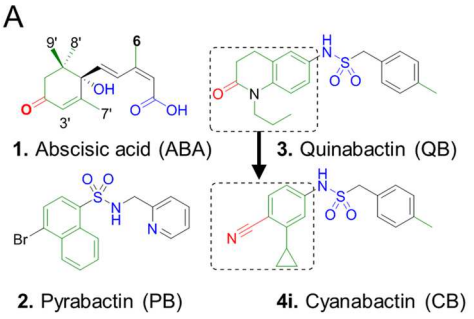
latter of which regulate guard cell aperture and transpiration. The SnRK 2s belong to the AMP kinase superfamily;[17] thus, the ABA receptor/PP2C response module is a land-plant-specific mechanism that links water availability to stress activated kinase signaling.

Angiosperm ABA receptors cluster into three phylogenetically distinct clades,[6,18] of which two (subfamilies I and II) are present in the genomes of extant members of early diverging land plant lineages.[6] Biochemical characterization of *Arabidopsis* PYLs has shown that the receptor subfamilies possess distinct properties. In general, subfamily I and II receptors are monomeric high-affinity receptors, while subfamily III receptors are lower-affinity and mostly dimeric.[19,20] The synthetic ABA agonists identified to date preferentially activate subfamily III receptors, which has helped define these as potential new agrochemical target sites.[1] ABA receptors possess two highly conserved surface loops that are critical for ligand and PP2C binding, termed the "gate" and "latch," which connect $\beta3-\beta4$ and $\beta5-\beta6$, respectively. The flexible gate loop can adopt open and closed forms; its closed state is stabilized by interactions between ABA and the gate-latch region. When a ternary ABA–receptor complex forms with a PP2C, a conserved Trp in a clade A PP2C-specific recognition loop inserts between the gate and latch loops and "locks" the closed receptor conformer.[21] The Trp indole N forms a hydrogen bond to ABA's cyclohexenone ketone *via* a key water that is coordinated by backbone contacts to conserved residues in the gate and latch loops; engagement of the Trp lock improves agonist activity.[1,22]

We have previously used phenotype-based chemical genetic screens to discover pyrabactin **2** (PB), which inhibits seed germination (a classic ABA response) by activating PYR1. Although *pyr1* loss-of-function mutants are insensitive to pyrabactin, they display normal ABA sensitivity in seed germination due to genetic redundancy.[7] Pyrabactin is a weak PYR1, PYL1, and PYL5 agonist[7,22] and weak PYL2 antagonist.[23] The *in vivo* effects of pyrabactin are strongest in seeds, where PYR1's mRNA is highly expressed, but it has relatively modest activity in adult tissues.[7] This limitation motivated subsequent efforts to identify synthetic agonists that could be used to modulate transpiration. Quinabactin **3** (QB) was discovered using target-based small molecule screens[22] and is a broader spectrum agonist with ABA-like potency on subfamily III receptors.[22] Quinabactin, unlike pyrabactin, reduces transpiration and activates multiple vegetative ABA responses in multiple angiosperms. This difference may be due to its increased potency relative to pyrabactin and/or its action on additional subfamily III receptors. To disentangle these possibilities and better define the key cellular targets that regulate transpiration, we set out to design new probe molecules possessing quinabactin-like potency but with greater selectivity. Here, we report our design of cyanabactin, which preferentially activates PYR1 with low nanomolar potency. We show that cyanabactin treatments elicit multiple ABA responses in both seeds and adult tissues and that genetic removal of both *PYR1* and its closest paralog *PYL1*, which together form the IIIA receptor subclade, eliminates both cyanabactin and quinabactin effects. Thus, we have developed a potent and selective ABA receptor agonist and used it to demonstrate that subfamily IIIA receptors are critical target sites for manipulating transpiration.

RESULTS AND DISCUSSION

Design of a Potent Nitrile Based ABA Receptor Agonist. Both pyrabactin and quinabactin share common structural elements required for agonist activity (Figure 1A).



B

	Clade	ABA	CB	QB
PYR1	IIIA	111 ± 3.8	87 ± 3	85 ± 6.9
PYL1	IIIA	231 ± 13	656 ± 109	279 ± 28
PYL2	IIIB	39 ± 2.3	3266 ± 13	211 ± 41
PYL3	IIIB	16 ± 0.1	6712 ± 1761	935 ± 75
PYL5	II	18 ± 5	150 ± 31	121 ± 7.3

Figure 1. Design and *in vitro* characterization of cyanabactin. (A) Structures of known ABA receptor agonists and the design strategy used in current study. Red indicates functionality making a hydrogen bond with the "Trp-lock" water. Green indicates functionality making hydrophobic interactions with L87, F61, V163, I110, and A160 in PYR1. Blue indicates functionality making a hydrogen bond with Glu 94 in PYR1. (B) Chemical-dependent inhibition of HAB1 by ABA receptors. IC_{50} values (nM) were determined as described in the Supporting Information using 50 nM HAB1, 100 nM receptor, and multiple concentrations of compounds; errors indicate standard deviation of triplicate measurements.

Our design strategy was to synthesize an ABA mimic that preserves the sulfonamide linkage and 4-methylbenzyl substructure present in quinabactin but reduces complexity of its bulky dihydroquinolinone ring. Specifically, we hypothesized that an aryl ring appropriately decorated with a nitrile could replace the dihydroquinolinone ring in quinabactin as nitrile nitrogens are good hydrogen bond acceptors,[24] and similar bioisosteric carbonyl/nitrile replacements have been successfully explored for androgen receptor modulators.[25] Moreover, we anticipated that this modification could increase ligand efficiency, by reducing the number of heavy atoms in the scaffold, and increase aqueous solubility. We appended small hydrophobic substituents ortho to the nitrile on our scaffold in an attempt to form hydrophobic interactions with the 3′ tunnel, which normally interacts with ABA's 7′ methyl group and is important for ligand affinity.[26] Docking studies using representative members of the envisioned ligands supported this design strategy; however, these analyses also suggested that the new scaffold might preferentially activate PYR1, as an H-bond to E94 in PYR1 was predicted to be absent in compounds when docked into PYL2 (Figure S1).

On the basis of this design strategy, we coupled a series of substituted 4-cyanoanilines and 4-methylbenzylsulfonyl chloride to synthesize 15 analogs (see SI Methods for detailed synthesis) and tested the compounds for ABA agonist activity *in vitro* using established receptor-mediated PP2C inhibition assays against all subfamily III receptors (PYR1, PYLs 1–3). To facilitate tests for *in vivo* activity, we created a transgenic

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Arabidopsis strain in which an enhanced luciferase gene is driven by the strongly ABA-regulated *MAPKKK18* promoter (see SI Methods for details); this line was used to rapidly assess compound bioactivity in live seedlings. We observed that the synthesized analog compounds **4a**−**4o** generally had greater potency on PYR1 relative to other dimeric receptors, consistent with our docking predictions, and that small electron donating substitutions were preferred ortho to the nitrile (Table S1). Of the compounds tested, **4i** was of interest as it possessed high potency against PYR1 (Figure 1B), induced strong luminescence in our *pMAPKKK18-Luc+* reporter strain, and inhibited *Arabidopsis* seed germination (Table S1 and Figure S2). We therefore selected **4i** for further characterization and named it "cyanabactin" (CB). To profile CB's selectivity in more detail, we assayed its activity against 11 ABA receptors in comparison to both ABA and quinabactin (Figure 1B and Table S2). These data show that cyanabactin has modest activity against PYL5 and no demonstrable activity on other monomeric receptors tested.

We next investigated the thermodynamics of cyanabactin/PYR1 binding reactions using isothermal titration calorimetry (ITC). Previous ITC studies have shown that ABA binds to PYR1 with an endothermic binding profile and high (∼50−100 μM) apparent K_d, which is due, in part, to an enthalpic cost of ABA-induced dimer dissociation.[27] ABA-dependent changes in $^{1}H-^{15}N$ heteronuclear NOE values observed on PYL10 suggest the enthalpic penalty may be partially offset by increased receptor conformational entropy upon ligand binding.[28] The nanomolar K_d's observed in the ABA/PP2C sensing system are achieved by ligand-induced PP2C binding, which stabilizes gate closure and likely lowers ligand K_{off}.[9] In contrast to published ABA-PYR1 isotherms, CB-PYR1 binding reactions display an exothermic enthalpy of −2 kcal/mol, a ΔS of 18.3 cal/mol K, and an apparent dissociation constant of 3.4 μM (308 K; Figure 2A and B). These values are comparable to those measured between ABA and high-affinity monomeric receptors.[27] Ligand efficiency, which is the ratio of the Gibbs free energy of the binding to the number of non-hydrogen ligand atoms, is a useful metric that can guide ligand optimization campaigns toward high affinity ligands with simplified scaffolds.[29] On the basis of our data, CB displays a favorable "drug-like" ligand efficiency of 0.33 kcal/mol/atom. Thus, the bulky bicyclic ring structures present in quinabactin and pyrabactin are not necessary for potent PYR1 agonists, which enables a more compact synthetic scaffold than those previously described.

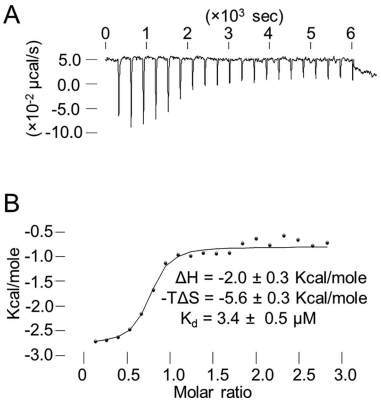

Figure 2. Isothermal titration measurements for CB with PYR1. (A) Representative thermogram for isothermal titration calorimetric analysis of binding of CB to PYR1. (B) Binding isotherm generated from corresponding data fitted to one-site binding model, with binding stoichiometry as n = 1.02 ± 0.045 sites.

To gain insight into the structural basis for cyanabactin's agonist activity, we solved the structure of a $PYR1^{1-181}$:cyanabactin complex using X-ray crystallography at a resolution of 1.63 Å (Table S3). The $PYR1^{1-181}$:CB complex crystallized in the space group *F*222 and contained a single protomer in the asymmetric unit. Several rounds of structural refinement were carried out prior to modeling cyanabactin into the ligand binding pocket's unbiased electron density (Figure 3A and B). Additionally, we confirmed our ligand placement using anomalous X-ray scattering from cyanabactin's sulfonamide sulfur atom (Figure S3). A real space correlation coefficient of 0.95 calculated between the unbiased electron density and cyanabactin indicates good agreement between the model and observed electron density. A second crystal structure was obtained at a resolution of 1.93 Å for analog **4m**, and this displayed a consistent ligand placement (Figure S3). The interactions between cyanabactin and PYR1 are similar to ABA−PYR1 interactions in a number of important ways (Figure 3 and Figure S4). First, the nitrile nitrogen forms a hydrogen bond with a water molecule that is stabilized by H-bonds to backbone amides of P88 and R116; this mimics the interactions between ABA's ketone and the PP2C's "tryptophan-lock" hydrogen bond network that aids in gate closure and PP2C binding; this validates a key feature of our design strategy. Second, the methyl group in the 4-methylbenzyl substructure of cyanabactin superimposes well with ABA's C6 methyl, which is an important structural requirement as ABA analogs devoid of this group have been found to be inactive *in vivo*.[30] Third, consistent with our docking studies, the sulfonamide NH in cyanabactin hydrogen bonds to E94, which normally forms a water-mediated H-bond to ABA's C1′-hydroxyl group. Fourth, the CB's cyclopropyl group extends into a hydrophobic solvent-exposed pore named the 3′ tunnel, which is normally occupied by ABA's C7′-methyl (Figure 3C−F). Our initial docking predictions favored a direct H-bond between a sulfonamide oxygen and K59, but in the crystal structure we observe that a sulfonamide oxygen forms water-mediated H-bonds to H60 and N167, which the docking method we used could not capture without *a priori* knowledge of the water's position. Thus, our X-ray crystallographic data demonstrate that cyanabactin engages PYR1 in a way that mimics key ABA-receptor contacts and is largely consistent with the docking predictions.

Cyanabactin Selectivity Is Determined by I110 and Avoids PYL2 Antagonism. The selectivity of CB for subfamily IIIA receptors is reminiscent of pyrabactin's selectivity. Between PYR1 and PYL2, the residues I110 and V114, respectively, which are homologous, are positioned proximal to ABA's 8′- and 9′-methyl groups (Figure 3), and the reduced pocket volume of PYR1 due to the extra methyl group influences responses to both pyrabactin and phaseic acid.[31,32] PYL2's larger pocket volume also enables pyrabactin to bind PYL2 in a nonproductive orientation that antagonizes receptor function and complicates the interpretation of biological activities observed using pyrabactin.[23,31,33] Given this, we tested CB's agonist activity against $PYR1^{I110V}$ and $PYL2^{V114I}$, which swap this homologous residue. These experiments show that CB is approximately 9-fold less active against $PYR1^{I110\ V}$ as compared to PYR1, whereas it is 3.5-fold more active on $PYL2^{V114I}$ relative to PYL2 (Figure S5 and Table S5), indicating

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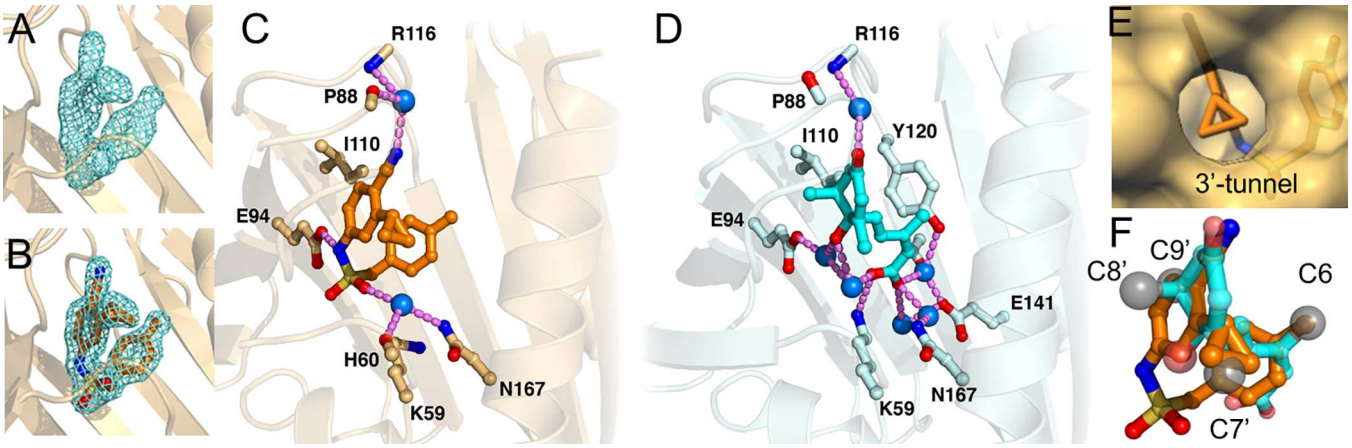

Figure 3. Unbiased electron density in the PYR1 binding pocket. (A) The F_o-F_c density in PYR1's ligand binding pocket after several rounds of refining the PYR11−181: cyanabactin structural model in the absence of cyanabactin. (B) The envelope of the unbiased electron density allowed us to unambiguously place cyanabactin in the structural model. Correct placement of cyanabactin is supported by a real space correlation coefficient of 0.95 calculated between the unbiased density and the modeled cyanabactin. (C) Network of water-mediated interactions with CB in the PYR1−CB complex (coordinates generated in this study). (D) Network of water-mediated interaction with ABA in the PYR1−ABA−HAB1 complex (PDB: 3K3K). (E) PYR1 surface displaying the cyclopropyl ring of CB extending into the 3′-tunnel. (F) Overlay of binding modes of ABA and CB in PYR1. Water molecules and hydrogen bonds shown as blue circles and pink dotted lines, respectively.

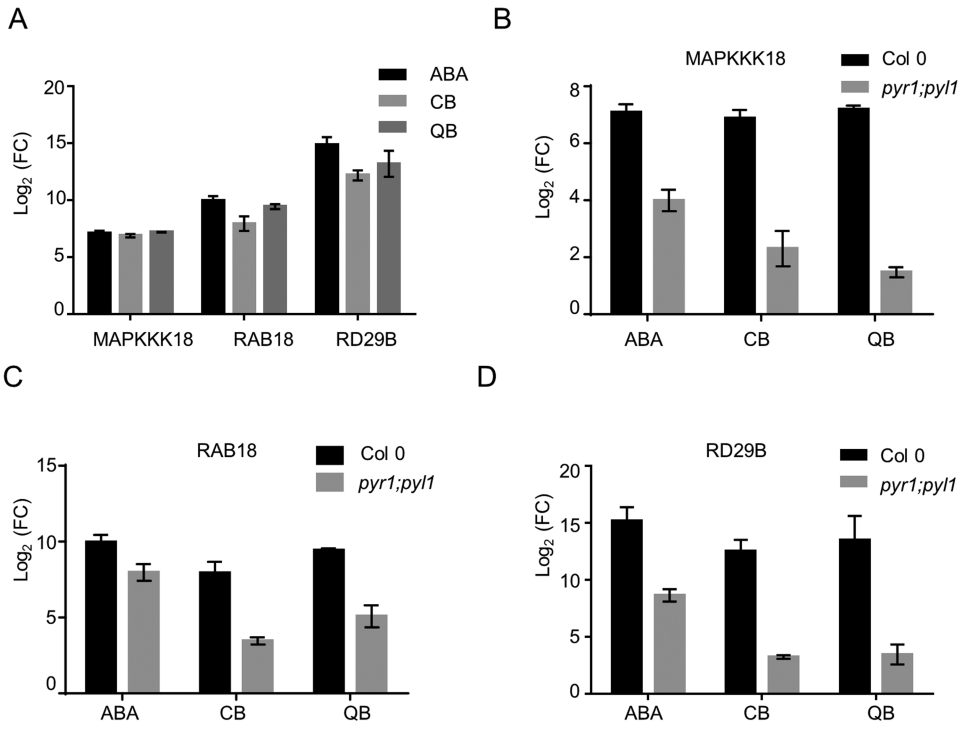

Figure 4. Regulation of ABA gene expression *via* PYR1/PYL1 receptors by CB. (A) ABA-responsive gene induction followed by qPCR 6 h after 25 μM, ABA or CB treatment. Expression levels are normalized to the constitutive gene expression of *PEX4*. (B−D) Comparison of transcript level for MAPKKK18, RAB18, and RD29B induced by individual ligands in Col 0 and *pyr1;pyl1* mutant.

that CB's selectivity for PYR1 is mediated in part by the I110/V114 position. Other positions are likely to also contribute, since PYL3 possesses an Ile in the homologous position and is less sensitive to CB than PYR1. We also tested CB for antagonism of ABA-mediated activation of PYL2, which we did not observe (Figure S5), indicating that CB is unlikely to have the multiple binding modes observed for pyrabactin; thus, unlike pyrabactin, CB is not a PYL2 antagonist. Collectively, our data show that CB's selectivity is due, in part, to differences in ligand binding caused by steric constraints that favor CB−PYR1 interactions and that CB does not suffer from the antagonism of PYL2 that complicates interpretation of biological data obtained using pyrabactin.

Cyanabactin Acts through Subfamily IIIA ABA Receptors. It remains unclear to which degree coactivation of multiple ABA receptors is necessary for robust ABA activity; CB's selectivity for PYR1 could potentially limit its utility as a tool for modulating adult plant ABA responses. We therefore examined CB's effects on seed germination and the *MAPKK18::Luc+* marker line. These data show that, in spite

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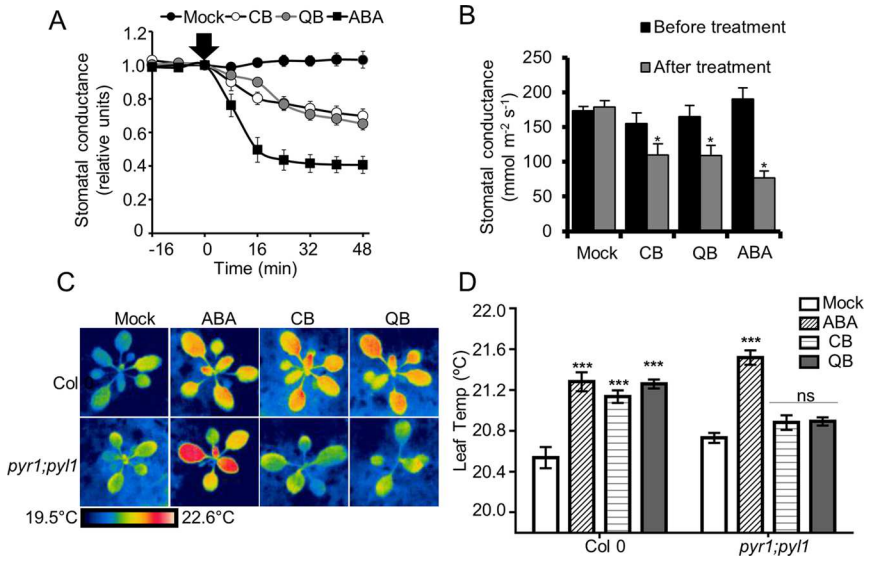

Figure 5. Reduction of transpiration in wild type *Arabidopsis thaliana* by CB. Comparison of cyanabactin (CB), quinabactin (QB), and ABA-induced stomatal closure in *Arabidopsis* Col-0 wild type plants. (A) Changes in whole plant stomatal conductance after spraying plants with CB, QB (25 μM), ABA (10 μM), and Mock (0.1% DMSO and 0.02% Silwet L77) at time 0 (indicated by the arrow). The values are shown as relative to the stomatal conductance at time 0 (n = 5 for mock, CB, and ABA, n = 8 for QB). (B) Stomatal conductance before and 48 min after the treatments described in A. Statistically significant effects of the treatments are shown by asterisks (repeated measures ANOVA with Tukey posthoc test). (C) Wild type *Arabidopsis* plants were sprayed with 50 μM compounds and imaged by thermography 16 h post treatment and quantified in D, and * indicates $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

of its selectivity, CB possesses clear ABA-like effects: it inhibits seed germination at 5 μM and activates luminescence in a *pMAPKKK18::Luc*$^+$ reporter strain (Figure S2). We next used a series of receptor mutant strains to dissect the roles of different ABA receptors in mediating CB's effects. To investigate its effects on ABA-regulated gene expression, we used quantitative PCR analyses of agonist treated wild type 10 day old seedlings and genotypes that remove CB's measurable target sites (*pyr1*, *pyl1*, *pyl2*, *pyl3*, and *pyl5*) and included comparisons to QB and ABA. These experiments demonstrate that CB treatments increase MAPKKK18, RAB18, and RD29B mRNA levels comparably to ABA and QB treatments (Figure 4A), indicating that CB has ABA-like effects on multiple ABA-regulated marker genes. These experiments also demonstrate that PYR1 and PYL1 are key targets *in vivo*, as the effects of both CB and QB are reduced by ∼95% in a *pyr1;pyl1* double mutant strain (Figure 4B–D and Figure S6). Thus, synthetic agonist-mediated activation of PYR1 and PYL1 is sufficient to alter levels of multiple ABA-regulated mRNAs. The data also suggest that subfamily IIIA receptors mediate a majority of ABA's effects on transcription as MAPKKK18 mRNA levels are reduced ∼80% in the *pyr1/pyl1* double mutant. Analyses of responses in *pyl2* and *pyl3* mutant strains indicate that neither of these receptors mediates the effects of our synthetic agonists or ABA in gene induction under the conditions tested (Figure S7). While it is clear from other experimental data that successive removal of receptors in higher-order mutant strains reduces ABA-induced transcriptional responses much more dramatically than the *pyr1/pyl1* double mutant,[34] our data nonetheless point to subfamily IIIA receptors as quantitatively major regulators of ABA-transcriptional responses in *Arabidopsis* seedlings. We further observe that a *pyr1;pyl1* double mutant displays greatly reduced seed sensitivity to CB and QB (Figure S8 and Table S6), demonstrating that their effects on germination are primarily mediated by subfamily IIIA receptors.

We next investigated the effects of CB, QB, and ABA on whole plant stomatal conductance (Figure 5A and B) using gas exchange measurements. These experiments reveal that CB treatments significantly reduce stomatal conductance comparably to QB treatments. In these assays, which were conducted immediately after agonist treatments, ABA has a stronger effect than either synthetic agonist; this may be due to differences in cellular uptake between the agonists and ABA or to the greater number of receptors that ABA activates. We also performed quantitative thermal imaging of *Arabidopsis* plants, which sensitively reports increases in leaf temperature due to reduced evaporative cooling when transpiration is reduced by ABA or other agonist treatments.[35] Cyanabactin treatments elicited increases in leaf temperature that are indicative of reduced transpiration and agonism of guard cell ABA receptors. Given the importance of subfamily IIIA receptors in mediating agonist transcriptional responses, we performed quantitative thermal imaging of agonist effects on the wild type and the *pyr1;pyl1* double mutant genotype. These experiments (Figure 5C and D) show that the leaf temperature increases induced by both CB and QB are abolished in a *pyr1;pyl1* mutant strain; however, ABA-induced responses remain intact. ABA receptor mRNA levels reported in public data sets[36] show that PYR1 is the most highly expressed ABA receptor in seed mRNA data sets but is only the fourth most highly expressed receptor in guard cells (Table S7), with both PYL2 and PYL5 possessing higher mRNA levels than PYR1. Since PYL2, PYL3, and PYL5 are activated by CB and QB, we additionally examined agonist responses in *pyl2*, *pyl3*, and *pyl5* mutants (Figure S8), which were similar to the wild type, indicating that the effects of CB, QB, and ABA on transpiration do not critically depend on any of these receptors. Thus, removal of subfamily IIIA receptors prevents CB and QB action on both transpiration and transcriptional responses in *Arabidopsis*. Collectively, our data demonstrate that CB has ABA-like effects in multiple physiological processes and demonstrate the importance of

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subfamily IIIA receptors to the action of synthetic ABA agonists.

Conclusions. Cyanabactin is, to our knowledge, the first rationally designed ABA receptor agonist that does not possess a core ABA skeleton. Quinabactin and pyrabactin both contain a bicyclic ring system connected *via* a sulfonamide linker to an aryl-methylene unit. In contrast, cyanabactin possesses a simpler monocyclic ring system and its potent activity demonstrates that ABA receptor agonists can be achieved using a relatively simple scaffold. Furthermore, the data demonstrate that CB's nitrile moiety is an effective bioisostere of both quinabactin's dihydroquinolinone oxygen and ABA's cyclohexenone oxygen, which interact with the Trp-lock water and its interconnected hydrogen bond network. Last, our data demonstrate that alkyl substituents ortho to the nitrile improve agonist activity by mimicking ABA's C7′ methyl group and occupying the 3′ tunnel, which was previously shown to improve the activity of C3′-thioether ABA-derivatives.[26] These insights and the simplified scaffold we have developed should facilitate agonist and antagonist design efforts, as aryl-nitriles are synthetically more accessible than the dihydroquinolinone ring system of quinabactin.

Aside from its value as a new scaffold, CB has proven biologically illuminating. Our findings demonstrate that multiple ABA effects, including seed germination inhibition, changes in gene expression, and reduced transpiration, can be mimicked by CB. Unlike ABA, which activates all 14 ABA receptors, or QB, which activates all subfamily III receptors, CB preferentially activates subfamily IIIA receptors, which our data show are necessary for both CB's and QB's effects. These observations point to IIIA receptors as key target sites for chemically controlling transpiration, which is consistent with recent data showing that a Brachypodium PYR1 ortholog plays a nonredundant role in regulating transpiration.[37] Phylogenetic analyses indicate that subfamily III receptors are relatively recent evolutionary innovations that have, to date, been observed only in angiosperm genome sequences.[6] It has been proposed that ABA's role in rapidly controlling stomatal responses in response to water deficit arose in angiosperms, as fern and lycophyte stomata are relatively insensitive to ABA.[38] The concomitant emergence of subfamily III receptors with high stomatal ABA sensitivity may ultimately explain why subfamily IIIA receptors make such good antitranspirant target sites.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.7b00650.

Details of synthesis, experimental procedures, compound characterization, and other supplementary data including figures and tables (PDF)

Accession Codes

The atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession numbers 5UR5 and 5UR6.

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Notes

The authors declare the following competing financial interest(s): S.R.C. and A.S.V. are co-inventors on a provisional patent application that covers molecules described in this manuscript.

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