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# Bioinspired Synthesis of a Sedaxane Metabolite Using Catalytic Vanadyl Acetylacetonate and Molecular Oxygen

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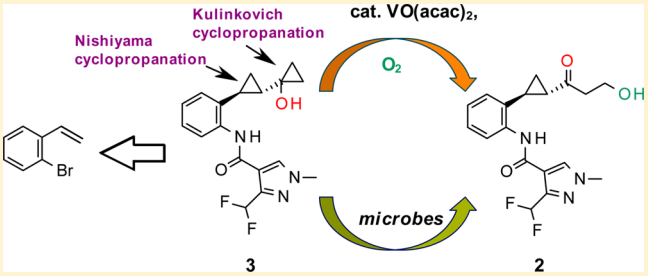
Supporting Information

**ABSTRACT:** A bioinspired synthesis of the sedaxane metabolite **2** from intermediate **3** using catalytic $VO(acac)_2$ and $O_2$ is described. Intermediate **3** was synthesized starting from 2-bromostyrene in four steps. The inner cyclopropyl ring of **3** was assembled with *trans* geometry using a highly diastereoselective Nishiyama cyclopropanation, and the outer hydroxycyclopropyl ring was installed using the Kulinkovich cyclopropanation. Additionally, conversion of **3** into **2** was demonstrated in *in vitro* microbial culture experiments consisting of bacteria and fungi.




## INTRODUCTION

Succinate dehydrogenase inhibitors (SDHIs) are an attractive class of fungicides used in agriculture for controlling fungal diseases of important agricultural crops. SDHIs inhibit the succinate dehydrogenase enzyme (located in the complex II respiration chain within the mitochondrial membrane), which is a functional part of the tricarboxylic acid cycle and linked to the mitochondrial electron transport chain.[1,2] Sedaxane (active ingredient in Vibrance fungicide) (**1**), a pyrazole carboxamide class of SDHI inhibitor, was recently registered as a seed treatment fungicide for cereal, canola, corn, potatoes, rice, sugar beets, sunflowers, and cotton crops (Figure 1).[3,4] Sedaxane demonstrates broad spectrum activity against a range of fungal pathogens within the *Ascomycetes* and *Basidiomycetes* classes of fungi.[3,4] In addition to its fungicidal activity, sedaxane promotes stronger and healthier roots, resulting in higher crop productivity. Sedaxane contains approximately 85% of the *trans* and approximately 13% of the *cis* isomers.

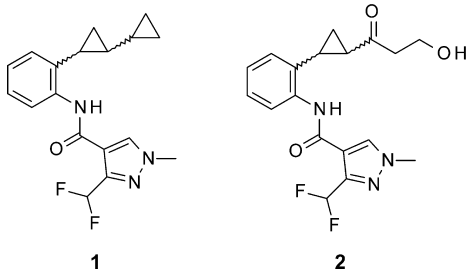
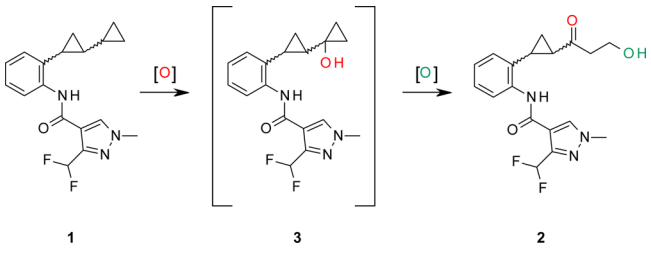



**Figure 1.** Sedaxane and related metabolite.

During the course of product development, we isolated metabolite **2** from crop studies (Figure 1). We were intrigued by the origin of **2** and became interested in understanding the biosynthetic pathway leading to its formation and also developing a synthetic route. We envisioned that sedaxane first underwent aerobic oxidation at the tertiary carbon of the outer cyclopropyl ring to form the tertiary cyclopropanol **3**, which presumably may be the primary metabolite of sedaxane. It is likely that the cyclopropanol **3** underwent further oxidation resulting in fragmentation of the outer cyclopropyl ring to form the *β*-hydroxyketone secondary metabolite **2** (Scheme 1). A literature search revealed Kirihara and co-workers had reported that bicyclo[*n*.1.0]alkanols **4** when reacted with molecular $O_2$ using catalytic vanadyl acetylacetonate in polar protic solvents such as EtOH (Scheme 2) formed a mixture of *β*-hydroxy ketones (major) and *β*-diketones (minor).[5,6] While this work was focused primarily on the bicyclo[*n*.1.0]alkanol substrates,

**Scheme 1. Proposed Biosynthetic Pathway of 2**

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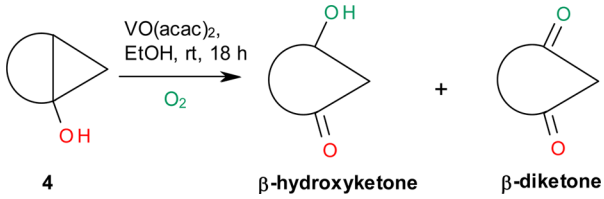
**Scheme 2. $VO(acac)_2$-Catalyzed Synthesis of $\beta$-Hydroxy Ketones from Bicyclo[*n*.1.0]alkanols**




we were pleased to find a close precedent that supports our hypothesis for the formation of $\beta$-hydroxy ketone **2** from intermediate **3**.

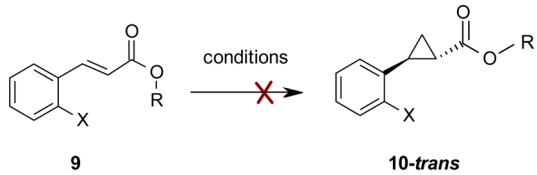
We focused our attention on developing a synthetic approach to access intermediate **3**. Retrosynthetic analysis suggested that **3** could be prepared by condensation of amino bicyclopropanol **5** with pyrazole acid chloride **6** (Scheme 3). Intermediate **5** could in turn be prepared from the arylcyclopropyl ester **7** via Kulinkovich cyclopropanation. Finally, **7** could be obtained by Nishiyama cyclopropanation of **8** or by either Corey–Chaykovsky or Simmons–Smith cyclopropanation of **9**.

## RESULTS AND DISCUSSION

Cyclopropanes are important motifs present in natural products and synthetic drug and agrochemical molecules.[7,8] Some notable examples include pyrethroid insecticides[8−10] and the quinolone class of antibiotics[8] (Ciprofloxacin and Sparfloxacin). The inherent rigidity of the cyclopropane ring has been employed to restrict the conformation of biologically useful molecules, which can sometimes lead to enhanced activity.[8,11] Despite the ubiquity of cyclopropanes in biologically active molecules, there were limited reports in the literature that described an asymmetric synthesis of *ortho*-substituted *trans*-1,2-disubstituted cyclopropanes.[12−15]

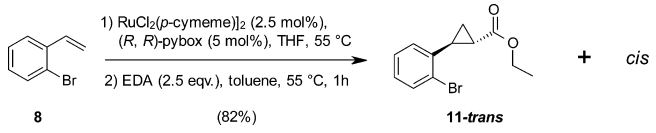
**I. Synthesis of Sedaxane Metabolite 2.** Our initial unsuccessful approach toward preparation of ***trans*-10** is outlined in Scheme 4. We focused our efforts on employing a cyclopropanation methodology that would generate a diastereomeric mixture, enriched in the *trans* isomer of **10**. We first attempted synthesis of ***trans*-10** using a stereospecific cyclopropanation of (*E*)-*o*-nitrocinnamic acid **9a** and the cinnamate **9b** using $Sm/CHI_3$.[16] However, for both substrates under these reaction conditions, we recovered only the starting material. We then attempted a diastereoselective palladium-catalyzed cyclopropanation of **9b** using diazomethane, but our efforts resulted in the recovery of the starting material.[17,18] Modified Corey–Chaykovsky cyclopropanation of **9b** using $(CH_3)_3S(O)I$/NaH

**Scheme 4. Attempted Approaches to Diastereoseletive Cyclopropanation**

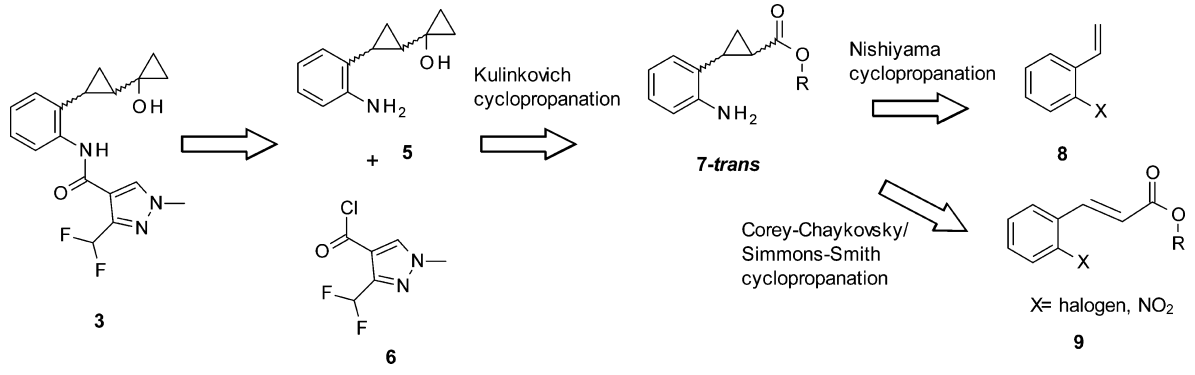



provided ***trans*-10** in only 15% yield.[19,20] Finally, Simmons–Smith cyclopropanation of **9b** and **9c** using diethyl zinc and diiodomethane yielded only the starting material.[21,22] While the attempts described above are effective methodologies for cyclopropanation, such methods were rendered ineffective possibly due to the steric effects of Br or an incompatibility of $NO_2$ group with reagents used in these methodologies. It is likely that in the case of Sm-promoted cyclopropanation the *o*-$NO_2$ group in **9** deactivates the reactive samarium carbenoid intermediate.[16] We were able to complete the cyclopropanation of *ortho*-substituted substrates by using metal-catalyzed asymmetric diazoacetate cyclopropanation[23,24] with excellent diastereocontrol. More specifically, we performed a Nishiyama cyclopropanation[25−27] using 2-bromostyrene **8** and ethyl diazoacetate (EDA) in the presence of catalytic Ru(II) and (*R*,*R*)-pybox to provide a 40:1 mixture of *trans*:*cis* cyclopropyl esters **11** (Scheme 5).[28] Interestingly, Nishiyama cyclopropanation under the same conditions using 2-chlorostyrene yielded a 50:1 *trans*:*cis* ratio in 89% yield. Athough cyclopropanation of 2-chlorostyrene provided a more desirable *trans*:*cis* isomer ratio and better yield as compared to 2-bromostyrene, we used **11** for further synthesis due to the wider range of methodologies available for amination of aryl bromides. We found Nishiyama cyclopropanation to be scalable and obtained a comparable ratio of *trans*- and *cis*-cyclopropyl esters at larger scales (70 mmol scale of styrene derivative).

**Scheme 5. Diastereoselective Nishiyama Cyclopropanation**




**Scheme 3. Retrosynthetic Analysis of 3**



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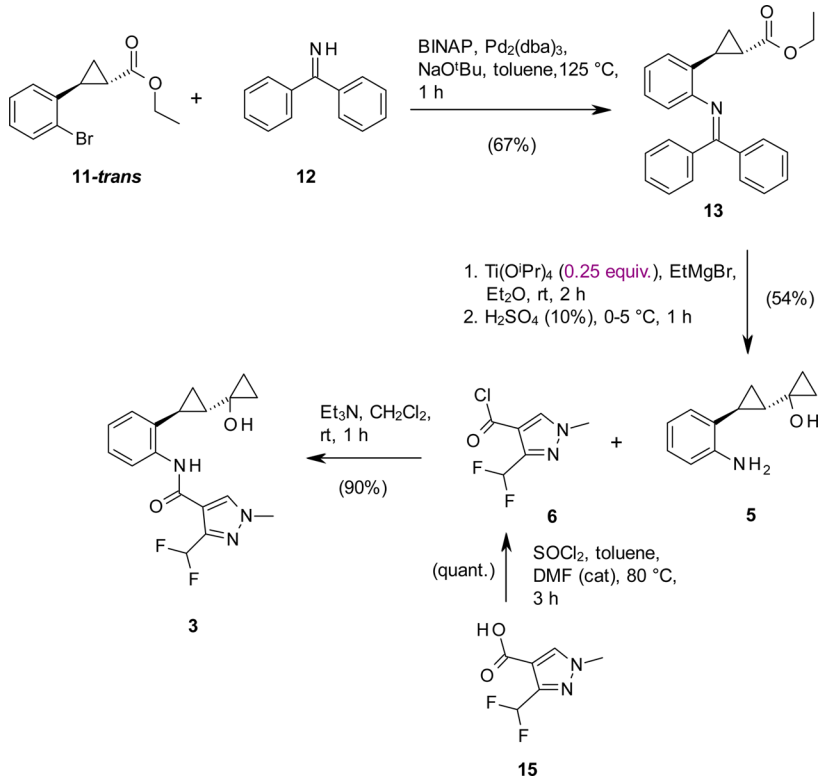
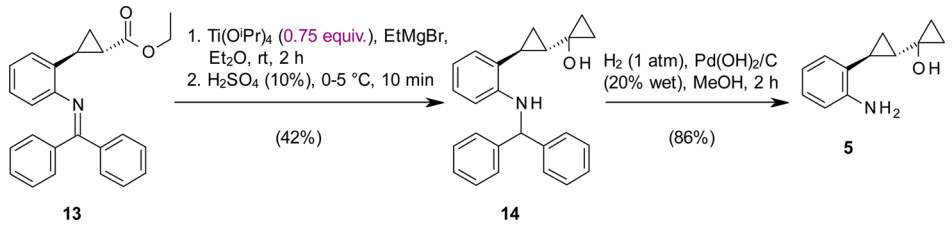
**Scheme 6. Synthesis of 3**




**Scheme 7. Kulinkovich Cyclopropanation Using Near-Stoichiometric Amounts of $Ti(O^iPr)_4$**




Our initial efforts toward amination of **11** using either ammonia or masked amines (phthalimide and triphenylsilyl amine) under Cu-[29] and Pd-catalyzed[30] conditions were unsuccessful. However, cyclopropyl ester **11** when reacted with diphenylimine **12** in the presence of catalytic Pd(0) using Buchwald's aryl amination conditions,[31] provided the protected cyclopropyl ester **13** (Scheme 6). It is noteworthy that under these reaction conditions with the possibility of epimerization at the 1-position in **13**, we observed only trace amounts (<1%) of the *cis* isomer. The cyclopropyl ester **13** was subjected to a two-step, one-pot reaction sequence involving Kulinkovich cyclopropanation[32,33] with ethylmagnesium bromide and catalytic titanium isopropoxide. This was followed by an *in situ* deprotection of the diphenylimine protecting group during the workup using 10% $H_2SO_4$ to form the amine **5**. The standard deprotection conditions for the removal of the diphenylimine protecting group requires refluxing in aqueous mineral acid (6 M HCl) for a few hours. We were pleasantly surprised to observe an *in situ* deprotection under the aqueous acidic workup conditions using 10% $H_2SO_4$. The Kulinkovich cyclopropanation reaction has a wide range of tolerance for varying amounts of titanium isopropoxide. However, we found that using 0.75 equiv of the reagent led to the reduction of the diphenylimine protecting group to form diphenylmethylamine **14** as the major product (Scheme 7).[34] Although **14** was an undesirable side product, we were able to convert it back to the desired product **5** by hydrogenation using catalytic $Pd(OH)_2/C$ in an 86% purified yield. As expected, we observed only trace levels (4−5%) of ring-opened side products. The Kulinkovich cyclopropanation of **13** using catalytic amounts of titanium isopropoxide (0.25 equiv) resulted in a cleaner reaction with no observed reduction of the diphenylimine. Next, the amine **5** was coupled with the pyrazole acid chloride **6** to form the desired product **3** in a 90% purified yield. The pyrazole acid chloride **6** was prepared by treatment of the pyrazole acid **15** with thionyl chloride and was used immediately without any further purification for the preparation of **3**.[35] We were pleased to find that intermediate **3** was stable as a solid at room temperature for an extended period of time and also in polar protic solvents like MeOH. However, we were surprised to observe a slow degradation of **3** in polar aprotic solvents like $CH_3CN$ and $CHCl_3$. Having completed the synthesis of **3**, we turned our attention to converting intermediate **3** to metabolite **2** using the methodology described by Kirihara.[5,6] To this end, **3** was treated with molecular oxygen (1 atm) in the presence of a catalytic amount of vanadyl acetylacetonate to obtain the metabolite **2** in 77% yield (Scheme 8). Interestingly, this reaction was scalable to 25 mmol with reproducible yield.



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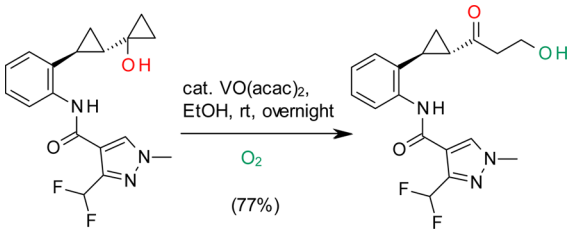
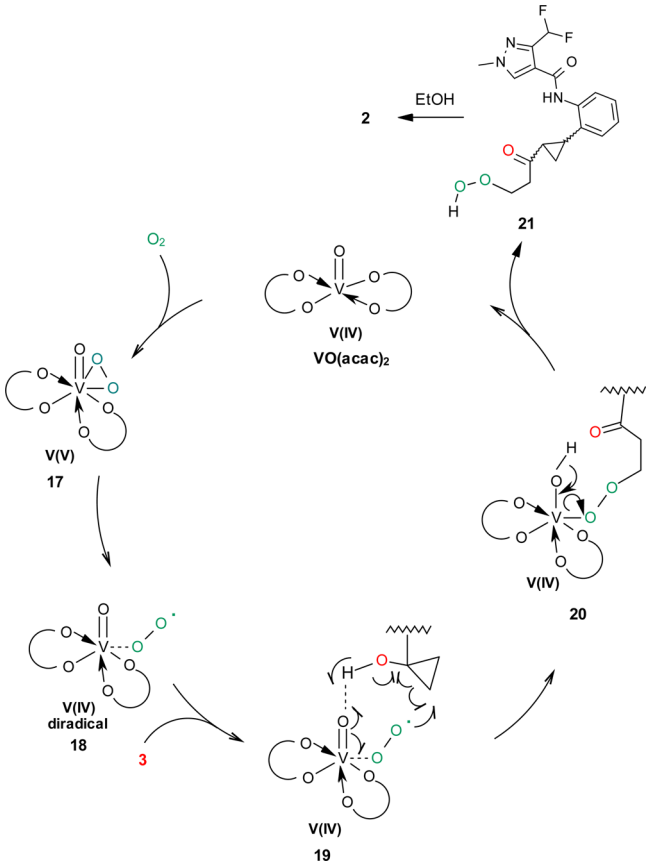
**Scheme 8. $VO(acac)_2$-Catalyzed Synthesis of 2**




**II. Mechanistic Proposal for the $VO(acac)_2$-Catalyzed Synthesis of 2.** The $VO(acac)_2$-catalyzed oxidation of bicyclo[*n*.1.0]alkanols **4** proceeds via the hydroperoxide intermediate **16** but the role of $V^{IV}$ in the mechanistic pathway was not discussed (Figure 2).[6] We propose that the first step in the oxidation of **3** to **2** involves insertion of $O_2$ into the $VO(acac)_2$, resulting in the activation of $O_2$ to generate the side-on bound peroxo-$V^V(\eta^2\text{-}O_2)$ intermediate **17** (Scheme 9). Formation of such a mononuclear, distorted pentagonal bipyramidal peroxo-$V^V(\eta^2\text{-}O_2)$ intermediate (upon treatment of $V^{IV}$ complexes with oxidants like $O_2$ and $H_2O_2$) has been confirmed by spectroscopic[36,37] and crystallographic[38−41] studies. The vanadium−oxygen bonds in the $V^V(\eta^2\text{-}O_2)$ group are not of a pure $\sigma$ character but rather composed of a fractional bond character due to the occupied three-centered four electron (3*c*4*e*) orbitals.[38] As a result, the oxidation state of vanadium in intermediate **17** is +5. Peroxo complex **17** undergoes homolytic cleavage to generate the diradical intermediate **18** in which the vanadium−oxygen bond is a single electron-shared bond.[41] Oxygen transfer from the diradical **18** to **3** is facilitated by hydrogen bonding between the apical oxygen and the alcohol **3** via transition state **19**. This transfer results in fragmentation and release of the cyclopropyl ring strain to generate intermediate **20**. Heterolytic cleavage in **20** results in the release of peroxide **21** and the regeneration of $V^{IV}$ for further catalysis. Finally, treatment of **21** with ethanol provides the metabolite **2**, with ethanol presumably acting as the reductant in the reaction.[42]

**III. Microbial Conversion of 3 into 2.** We were further interested in confirming our hypothesis for the biogenesis of metabolite **2** from the intermediate **3**. We conducted a microbial degradation screening experiment by incubating intermediate **3** in *in vitro* cultures consisting of various strains of bacteria and fungi (Table 1). After 10 days, the best conversion was observed in *Streptomyces lydicus* (17% formation of **2**, entry 5) followed by *Cunninghamella elegans* (10% formation of **2**, entry 4). Additionally, we believe that there may be other competing degradation products formed from intermediate **3** in the microbial culture which have not been characterized. The results strongly support our hypothesis that **3** is a biosynthetic precursor to **2**.

**Scheme 9. Plausible Mechanism for the Formation of Metabolite 2**




## CONCLUSION

In summary, a biomimetic synthesis of metabolite **2** from intermediate **3** has been accomplished using catalytic vanadyl acetylacetonate and molecular $O_2$. Additionally, intermediate **3** was converted to metabolite **2** using *in vitro* experiments, thus providing corroborating support for the biosynthesis of **2**. A plausible mechanism for the formation of **2** from **3** has been provided highlighting the role of vanadium in the catalytic cycle. The highly diastereoselective Nishiyama cyclopropanation of **8** afforded the desired *trans*-cyclopropyl ester **11** in a 40(*trans*):1(*cis*) ratio. The bioinspired synthesis of **2** may be extrapolated to $\beta$-hydroxy ketone type metabolites obtained from drug/agrochemical molecules containing the cyclopropyl ring motif. The use of catalytic vanadyl acetylacetonate at ambient temperature and molecular $O_2$ coupled with the scalability of this reaction makes this methodology attractive.

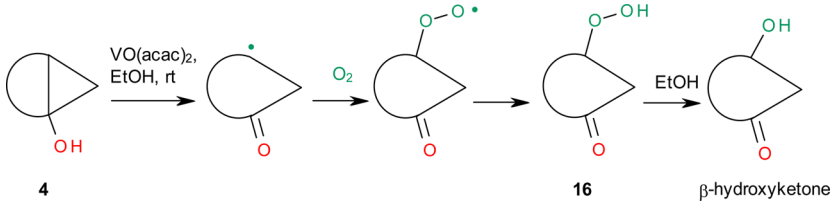



**Figure 2.** Mechanistic proposal by Kirihara and co-workers for the formation of $\beta$-hydroxy ketones from bicyclo[*n*.1.0]alkanols **4**.



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Table 1. Microbial Conversion of 3 into 2 after 10 Days[a]

| entry | organism type | species | % 3[b] remaining | % 2[b] formed | 3 μg/mL in well extract | 2 μg/mL in well extract |
|---|---|---|---|---|---|---|
| 1 | fungus | *Absidia coerulea (ATC 38187)* | 67 | <5 | 1.45 | 0.05 |
| 2 | fungus | *Aspergillus niger* (F 571) | 100 | <5 | 2.15 | 0.06 |
| 3 | fungus | *Beauveria bassiana (DMS 1344)* | 81 | 7 | 1.75 | 0.16 |
| 4 | fungus | *Cunninghamella elegans* (IMI 128924) | 65 | 10 | 1.40 | 0.22 |
| 5 | actinomyces | *Streptomyces lydicus (NRLL-2433)* | 81 | 17 | 1.75 | 0.37 |
| 6 | actinomyces | *Streptomyces tubercidicus (R-922)* | 93 | 9 | 2.00 | 0.19 |
| 7 | actinomyces | isolate unknown | 84 | 6 | 1.80 | 0.12 |
| | | blank control | 100 | <5 | 2.15 | 0.02 |

[a]LC−MS/MS quantification of metabolite **2** and intermediate **3**. [b]The % of **2** and **3** were calculated relative to the blank control concentration (2.15 μg/mL).

## EXPERIMENTAL SECTION

**General Methods.** Reagents and solvents were purchased from commercial suppliers and used without further purification. Solvents were dried on activated 4 Å molecular sieves. All reactions were performed under either a $N_2$ or Ar environment. The conversion of starting materials was monitored by either thin-layer chromatography (TLC) using silica gel plates (silica gel 60 F254, 0.25 mm), with components were visualized by observation under UV light (254 and 365 nm), or GCMS using chemical ionization mode using methane as a reagent gas. Melting points were measured by differential scanning calorimetry. High-resolution mass spectrometry (HRMS) measurements were obtained using positive electrospray ionization (ESI) quadrupole orbitrap instrumentation and were obtained in profile mode using the 140000 resolution setting. Dilute acetonitrile solutions of the compounds were infused at 0.5 μL/min into the mobile phase [50:50 acetonitrile:water (0.1% formic acid)] at a flow rate of 0.4 mL/min for which the ESI conditions had been optimized. $^1$H and $^{13}$C NMR spectra were recorded at 500 and 125 MHz, respectively. $^1$H NMR chemical shifts (in ppm) were referenced to protic impurity in $CDCl_3$ (7.27 ppm) or $CD_3CN$ (1.93 ppm). $^{13}$C NMR spectra were calibrated with $CDCl_3$ (77.23 ppm) or $CD_3CN$ (1.39 ppm). Multiplicities are indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); br s (broad singlet); dd (doublet of doublets); ddd (doublet of doublet of doublets); td (triplet of doublets); dt (doublet of triplets) etc. Coupling constants are reported in hertz. IR spectra were recorded on a FT-spectrometer and are reported in terms of frequency of absorption ($cm^{-1}$). Optical rotation data were obtained on a polarimeter and are reported in terms of degrees of rotation of plane polarized light at 589 nm.

Microbial assays were carried out in a Class II microbiological cabinet using sterile equipment and adhering to aseptic working procedures, except where indicated otherwise. Compounds **2** and **3** were each dissolved separately in $CH_3CN$ to give stock solutions of 500 μg/mL. Serial dilutions were performed to give 20, 10, 1, and 0.1 μg/mL standards in 10 and 50 mL volumetric flasks. ISP-2 media solution (500 mL) was prepared by dissolving 2 g of yeast extract, 2 g of glucose, and 5 g of Bacto Malt extract in 500 mL of Ultra Pure Water (UPW), and the resulting solution was sterilized in an autoclave. An adsorption test (to check for loss during filtration) was performed using 0.1 μg/mL solution of **2** and **3** in $CH_3CN$ and filtering through a Whatman Anotop 10, 0.2 μm 10 mm syringe filter. LC−MS/MS for quantification of metabolite **2** and intermediate **3** was performed using a turbospray ion source in negative mode. The ions monitored for the metabolite **2** were $m/z$ 362 → 332 and $m/z$ 362 → 91 and for the intermediate **3** were $m/z$ 346 → 91 and 346 → 131. HPLC was performed on an ACE 5-C18 column (50 × 3.0 mm) using a $CH_3CN$/UPW (0.05% acetic acid) gradient at 30 °C at a flow rate of 0.5 mL/min. The limit of quantification was 0.005 μg/mL.

*Ethyl (1S,2S)-2-(2-Bromophenyl)cyclopropanecarboxylate (**11**).* To a stirred solution of dichloro(*p*-cymene)ruthenium(II) dimer (1.03 g, 1.68 mmol) and (*R*,*R*)-pybox (1.02 g, 3.38 mmol) in anhydrous THF (90 mL) at rt was added a solution of 2-bromostyrene (11.90 g, 65.00 mmol) in anhydrous THF (45 mL). The mixture was heated at 55 °C, and a solution of ethyl diazoacetate (18.54 g, 162.48 mmol) in anhydrous toluene (100 mL) was added dropwise over a period of 3 h. The reaction was monitored by GCMS. After 1 h, 3% unreacted 2-bromostyrene was left in the reaction. The solution was cooled and quenched with a 10% solution of $CH_3CO_2H$ in water (25 mL). The organic layer was separated, washed with water (25 mL) and brine solution (25 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo to provide a dark reddish-brown oil. The crude product was purified by flash column chromatography on silica gel using [hexanes/EtOAc (20%)] to afford ***trans*-11** (12.79 g) as a clear colorless oil and a second fraction of ***trans*-11** and ***cis*-11** (1.60 g) mixture. The mixture was resubjected to chromatography to obtain ***trans*-11** (1.24 g) and ***cis*-11** (0.35 g) to afford combined ***trans*-11** (14.03 g) and ***cis*-11** (0.35 g) giving a ratio of 40(*trans*):(1)*cis*. The overall yield of ***trans*-11** and ***cis*-11** was 82%. The ***trans*-11** was used directly for the next step: $[\alpha]_D^{26}$ +84.8 (*c* 1.01, $CHCl_3$); $^1$H NMR ($CDCl_3$, 500 MHz) δ 7.58 (d, 1H, *J* = 7.8 Hz), 7.25 (t, 1H, *J* = 7.8 Hz), 7.11 (t, 1H, *J* = 7.8 Hz), 7.04 (d, 1H, *J* = 7.8 Hz), 4.20 (m, 2H), 2.73 (ddd, 1H, *J* = 9.1, 6.8, 4.5 Hz), 1.81 (dt, 1H, *J* = 8.4, 4.5 Hz), 1.65 (m, 1H), 1.34 (m, 1H), 1.32 (t, 3H, *J* = 7.5 Hz); $^{13}$C{$^1$H} NMR ($CDCl_3$, 125 MHz) δ 173.5, 139.3, 132.8, 128.4, 127.7, 127.6, 126.5, 60.9, 27.2, 23.3, 15.9, 14.5; IR (neat, $cm^{-1}$) 2980, 1722, 1178, 730; HRMS (ESI-Q-orbitrap) $m/z$ $[M + H]^+$ calcd for $C_{12}H_{14}BrO_2$ 269.01717, found 269.01626.

*Ethyl (1S, 2S)-2-[2-[(Diphenylmethylidene)amino]phenyl]-cyclopropanecarboxylate (**13**).* To a solution of ***trans*-11** (6.30 g, 23.40 mmol) in anhydrous toluene (50 mL) were added $Pd_2(dba)_3$ (0.050 g, 0.055 mmol), racemic-BINAP (0.122 g, 0.20 mmol), benzophenone imine **12** (4.45 g, 24.60 mmol), and NaO$^t$Bu (2.81 g, 29.25 mmol). The mixture was heated at 125 °C for 1.0 h. The solution was cooled to rt, filtered through Celite, and washed with EtOAc. The combined filtrate was concentrated in vacuo to give a brown oil. The crude product was purified by flash column chromatography on silica gel using [hexanes/EtOAc (10%)] to afford **13** (5.79 g, 67%) as a yellow oil: $[\alpha]_D^{28}$ +115.5 (*c* 0.40, $CHCl_3$); $^1$H NMR ($CDCl_3$, 500 MHz) δ 7.85−6.85 (m, 13H), 6.60 (d, 1H, *J* = 7.7 Hz), 4.08 (dq, 1H, *J* = 7.2, 10.7 Hz), 3.93 (dq, 1H, *J* = 7.2, 10.7 Hz), 2.45 (m, 1H), 1.81 (ddd, 1H, *J* = 4.6, 5.1, 8.4 Hz), 1.51 (ddd, 1H, *J* = 4.6, 5.2, 9.4 Hz), 1.31 (ddd, 1H, *J* = 4.6, 6.8, 8.4 Hz), 1.15 (t, 3H, *J* = 7.2 Hz); $^{13}$C{$^1$H} NMR ($CDCl_3$, 125 MHz) δ 173.9, 168.0, 143.9, 139.2, 136.4, 131.1, 130.3, 129.8, 129.7, 129.2, 129.0, 128.5, 128.4, 128.1, 126.9, 126.0, 123.6, 120.6, 60.7, 23.5, 23.1, 15.9, 14.3; IR (neat, $cm^{-1}$) 2979, 1720, 1176, 746; HRMS (ESI-Q-orbitrap) $m/z$ $[M + H]^+$ calcd for $C_{25}H_{24}O_2N$ 370.18106, found 370.18002.

*Ethyl (1S,2S)-2-[2-[(Diphenylmethyl)amino]phenyl]-cyclopropanecarboxylate (**14**).* A solution of **13** (7.65 g, 20.70 mmol) in anhydrous ethyl ether (75 mL) was cooled at 0 °C. To this was added Ti(O$^i$Pr)$_4$ (4.46 g, 15.68 mmol) followed by dropwise addition of an EtMgBr solution (3 M in $Et_2O$, 19.80 mL, 59.40 mmol) over 30 min. The solution was stirred at 0−5 °C for 2 h and then warmed to rt and stirred for an additional 2 h. The solution was cooled at 0 °C, and a 10% aq $H_2SO_4$ solution (70 mL) was added slowly over 10 min to maintain the internal temperature between 0 and 5 °C. The solution was stirred for 15 min, and EtOAc (50 mL) was added. The organic layer was separated, washed with satd $NaHCO_3$ solution (60 mL), water (60 mL), and brine (50 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo to give a yellow oil. The crude product was


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purified by flash chromatography on silica gel using [hexanes/EtOAc (15%)] to afford **14** (3.05 g, 42%) as a light yellow oil: $[\alpha]_D^{27}$ −51.2 (*c* 0.47, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 7.28−7.46 (m, 10H), 7.12 (m, 1H), 7.05 (m, 1H), 6.69 (t, 1H, *J* = 7.3 Hz), 6.48 (d, 1H, *J* = 7.3 Hz), 5.65 (s, 1H), 5.44 (br, 1H), 1.78 (dt, 1H, *J* = 5.3, 8.8 Hz), 1.40 (dt, 1H, *J* = 5.1, 8.8 Hz), 1.49 (br, 1H), 0.97 (ddd, 1H, *J* = 5.2, 5.3, 8.8 Hz), 0.75 (dt, 1H, *J* = 5.1, 8.8 Hz), 0.40−0.73 (m, 4H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz) $\delta$ 147.0, 143.3, 142.9, 128.9, 128.9, 128.8, 128.2, 127.8, 127.7, 127.6, 127.4, 125.4, 116.8, 111.0, 63.1, 56.8, 25.5, 17.8, 13.7, 11.9, 10.1; IR (neat, $cm^{-1}$) 3424, 3370, 3024, 1505, 1450, 746; HRMS (ESI-Q-orbitrap) *m*/*z* $[M + H]^+$ calcd for $C_{25}H_{26}NO$ 356.20089, found 356.20013.

*(1′S,2′S)-2′-(2-Aminophenyl)-1,1′-bi(cyclopropyl)-1-ol (**5**) (from **13**).* A solution of **13** (5.79 g, 15.67 mmol) in anhydrous ethyl ether (100 mL) was cooled at 0 °C. To this was added $Ti(^iOPr)_4$ (1.11 g, 3.92 mmol), followed by dropwise addition of an EtMgBr solution (3 M in $Et_2O$, 10.5 mL, 31.34 mmol) over 30 min. The ice bath was removed, and the solution was warmed to rt and then stirred for 2 h. The solution was cooled to 0 °C, and a 10% aq $H_2SO_4$ solution (100 mL) was added slowly over 10 min to maintain the internal temperature between 0 and 5 °C. The solution was stirred for 1 h and ethyl ether (50 mL) was added. The organic layer was separated and extracted twice with 10% $H_2SO_4$ solution (50 mL). The combined aqueous layer was basified with 2 M NaOH solution (pH = 9) and extracted with EtOAc (3 × 60 mL). The organic layer was washed with water (50 mL) and brine solution (50 mL), dried ($Na_2SO_4$), filtered, and concentrated in vacuo to give a dark brown oil. The crude product was purified by flash chromatography on silica gel using [hexanes/EtOAc (35%)] to afford **5** (1.61 g, 54%) as light brown colored viscous oil. This was used without any further purification for the next step. $[\alpha]_D^{27}$ −30.3 (*c* 0.64, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 7.06 (t, 1H, *J* = 7.7 Hz), 7.02 (d, 1H, *J* = 7.3 Hz), 6.66−6.74 (m, 2H), 3.78 (br, 3H), 1.73 (dt, 1H, *J* = 5.5, 8.7 Hz), 1.43 (dt, 1H, *J* = 5.2, 8.7 Hz), 0.45−0.96 (m, 6H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz) $\delta$ 146.3, 128.6, 127.6, 126.2, 118.4, 115.1, 57.2, 25.7, 18.0, 13.8, 11.2, 9.9; IR (neat, $cm^{-1}$) 3360, 1497, 1454, 1214, 928, 745; HRMS (ESI-Q-orbitrap) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{16}ON$ 190.12264, found 190.12228.

*(1′S,2′S)-2′-(2-Aminophenyl)-1,1′-bi(cyclopropyl)-1-ol (**5**) (from **14**).* To a solution of **14** (2.61 g, 7.35 mol) in methanol (60 mL) was added $Pd(OH)_2/C$ (Pearlman's catalyst, 0.120 g, 20% wt, wet). Hydrogen gas (1 atm, via balloon) was bubbled into the solution for 1 h. The solution was filtered on Celite and washed with MeOH. The combined filtrate was concentrated in vacuo to give a brown oil. The crude product was purified by flash column chromatography on silica gel using [hexanes/EtOAc (40%)] to afford **5** (1.20 g, 86%) as a brown oil. The analytical data of compound **5** obtained was identical to that obtained from compound **13**. This was used without any further purification for the next step.

*3-(Difluoromethyl)-1-methyl-1H-pyrazole-4-carbonyl Chloride (**6**).* To a suspension of acid **15** (1.20 g, 6.81 mmol) in anhydrous toluene (10 mL) was added thionyl chloride (16.21 g, 136.27 mmol), and the solution was heated at 90 °C for 3 h. During the course of heating, all of the solids dissolved and the solution became clear. The solution was cooled at rt and concentrated in vacuo to afford a yellow oil. The crude product was azeotroped with toluene (25 mL) and heptane (25 mL) to provide **6** (0.34 g, quant) as a yellow oil which was used immediately without further purification for the next step: $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 8.08 (s, 1H), 6.92 (t, 1H, $J_{F,H}$ = 53.3 Hz), 4.01 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 75 MHz) $\delta$ 158.3, 146.7 (t, $J_{F-C}$ = 16 Hz), 139.2, 117.4, 108.9 (t, $J_{F-C}$ = 238 Hz), 40.3.

*3-(Difluoromethyl)-N-[2-[(1S,2S)-1′-hydroxy-1,1′-bi(cyclopropyl)-2-yl]phenyl]-1-methyl-1H-pyrazole-4-carboxamide (**3**).* To a solution of amine **5** (0.35 g, 1.86 mmol) and triethylamine (0.74 g, 7.12 mmol) in anhydrous $CH_2Cl_2$ (4 mL) was added a solution of acid chloride **6** (0.34 g, 1.78 mmol) in anhydrous $CH_2Cl_2$ (5 mL) dropwise. The solution was stirred at rt for 1.5 h. The solution was concentrated in vacuo and the crude product was purified by flash column chromatography on silica gel using [hexanes/EtOAc (45%)] to provide **3** (0.55 g, 90%) as a white solid: mp = 146−149 °C; $[\alpha]_D^{26}$ −25.5 (*c* 1.03, MeOH); $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 8.67 (b, 1H), 8.12(s, 1H), 7.95 (d, 1H, *J* = 7.7 Hz), 7.18 (t, 1H, *J* = 8.1 Hz), 7.16 (t, 1H, $J_{F,H}$ = 54.3 Hz), 7.06 (t, 1H, *J* = 8.1 Hz), 7.02 (d, 1H, *J* = 7.7 Hz), 3.86 (s, 3H), 3.41 (br, 1H), 1.84 (dt, 1H, *J* = 5.4, 8.8 Hz), 1.45 ppm (dt, 1H *J* = 5.4, 8.6 Hz), 0.36−1.08 (m, 6H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125 MHz) $\delta$ 160.6, 145.3 (t, $J_{F-C}$ = 25.4 Hz), 136.9, 133.9, 132.7, 126.9, 126.8, 125.2, 123.0, 116.8, 110.7 (t, $J_{F-C}$ = 234.4 Hz), 57.2, 39.7, 27.9, 18.2, 13.7, 10.3, 10.0; IR (neat, $cm^{-1}$) 3351, 3287, 1525, 1216, 1014, 770; HRMS (ESI-Q-orbitrap) *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{20}O_2N_3F_2$ 348.15181, found 348.15120.

*3-(Difluoromethyl)-N-[2-[(1S,2S)-2-(3-hydroxypropanoyl)-cyclopropyl]phenyl]-1-methyl-1H-pyrazole-4-carboxamide (**2**).* To a solution of **3** (0.55 g 1.60 mmol) in anhydrous ethanol (25 mL) was added vanadyl acetylacetonate (0.085 g, 0.32 mmol). The solution was stirred under an $O_2$ atmosphere (1 atm) overnight. Saturated $NaHCO_3$ solution (20 mL) and EtOAC (50 mL) were added, and the mixture was stirred for 5 min. The precipitate was filtered and washed with EtOAc. The combined filtrate was washed with brine solution, dried ($Na_2SO_4$), filtered, and concentrated in vacuo to give a yellow oil. The crude product was purified by flash column chromatography on silica gel using [$CH_2Cl_2/CH_3CN$ (20%)] to provide **2** (0.45 g, 77%) as a colorless oil which solidified at rt: mp = >228 dec; $[\alpha]_D^{28}$ +128.9 (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CD_3CN$, 500 MHz) $\delta$ 8.27 (br, 1H), 8.00 (s, 1H), 7.53 ppm (d, 1H, *J* = 7.7 Hz), 7.26 (t, 1H, *J* = 7.7 Hz), 7.22 (t, 1H, $J_{F,H}$ = 54.0 Hz), 7.20 (t, 1H, *J* = 7.7 Hz), 7.16 (d, 1H, *J* = 7.7 Hz), 3.93 (s, 3H), 3.76−3.64 (m, 2H), 2.88 (t, 1H, *J* = 5.5 Hz), 2.72 (ddd, 1H, *J* = 5.3, 6.1, 16.4 Hz), 2.67 (ddd, 1H, *J* = 5.4, 6.4, 16.4 Hz), 2.48 (ddd, 1H, *J* = 4.4, 6.8, 9.0 Hz), 2.17 (ddd, 1H, *J* = 4.4, 5.2, 8.2 Hz), 1.50 (ddd, 1H, *J* = 4.0, 5.2, 9.0 Hz), 1.41 (ddd, 1H, *J* = 4.0, 6.8, 8.2 Hz); $^{13}C\{^1H\}$ NMR ($CD_3CN$, 125 MHz) $\delta$ 209.4, 161.4, 146.4 (t, $J_{F-C}$ = 23.4 Hz), 137.7, 135.4, 133.4, 128.2, 127.7, 127.2, 126.7, 117.4, 111.2 (t, $J_{F-C}$ = 233.3 Hz), 58.3, 46.8, 40.3, 31.6, 25.3, 17.2; IR (neat, $cm^{-1}$) 3423, 3270, 1643, 1546, 1026, 758; HRMS (ESI-Q-orbitrap) *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{20}O_3N_3F_2$ 364.14672, found 364.14624.

**General Procedure for Microbial Conversion of 3 to 2.** Compound **3** (1 mL, 500 μg/mL in $CH_3CN$) was added to 50 mL of ISP-2 media in a sterile 250 mL bottle to give a solution concentration of 10 μg/mL of **3**. One vial of each of the fungal and bacterial strains (listed in Table 1) was removed from the −80 °C freezer and allowed to defrost and warm to room temperature. An aliquot of 1.5 mL of treated ISP-2 solution (containing 15 μg of **3**) prepared above was added to the required number of wells in the well plate. Each well was then inoculated with an individual strain of fungus and bacteria (listed in Table 1) using a fresh sterile inoculating loop for each application. The plate covers were fixed in place and the plates were placed onto the shaker in a room at 25 °C and 50% relative humidity. The plates were shaken at a speed enough to allow sufficient aeration without splashing and subsequent cross contamination. From this point onward, work was conducted in the regular laboratory without the need for sterile equipment. After 10 days, the plate was removed from the shaker, and the entire contents of each well were carefully transferred to plastic disposable centrifuge tubes (15 mL size). The wells were rinsed with 2 × 1 mL of MeOH and the rinse added to the tubes. The final volume was adjusted to 6 mL and shaken briefly to mix thoroughly. Samples were centrifuged at 4000 rpm for 5 min, and the resulting supernatants were stored frozen at −80 °C for 40 days. Samples were allowed to defrost, and aliquots were filtered through anatop filters into snap top vials. Appropriate dilutions for compounds **2** and **3** were prepared by withdrawing aliquots of 20 μL (for analysis of **3**) and 100 μL (for analysis of **2**) of the filtrate, and each was diluted to 1 mL with mobile phase [$CH_3CN$/UPW (10:90)] in an HPLC vial. The concentration of **2** and **3** were analyzed by LCMS−MS against the control sample and the mixed standards (0.1−0.01 μg/mL concentration) in $CH_3CN$/UPW (10:90). Samples were screened for the loss of **3** and the formation of metabolite **2**.



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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01700.

$^{1}$H and $^{13}$C NMR spectra for all new compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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