SUPPORTING INFORMATION

On the Way to Potential Antifungal Compounds: Synthesis and *in vitro* Activity of 2-benzofuranylacetic Acid Amides

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1. Experimental Procedures

1.1. General information

All the solvents were used as received from the supplier without any modifications. All reagents were used as received from commercial suppliers. Reactions progress was monitored by thin layer chromatography (TLC) performed on aluminium plates coated with silica gel F254 with 0.2 mm thickness TLC plates were visualized using ultraviolet (UV) light at 254 nm or stained with p-anisaldehyde, vanillin, ninhydrin, or phosphomolybdic acid solutions. Flash column chromatography was performed using silica gel 60 (230-400 mesh). Neat infrared spectra were recorded using a THERMO NICOLET-NEXUS (FT-IR) with PIKE MIRacle ATR cell. Wavenumbers (nmax) are reported in cm⁻¹. High-Resolution Mass spectrometry was recorded using an Agilent 5973 (70 eV) spectrometer using electrospray ionization (ESI). GC-MS was recorded in a Thermo Scientific Trace 1300. NMR spectra were recorded using a BRUKER Advance III HD Ascend 400 spectrometer. Chemical shifts are given in parts per million (ppm, δ), referenced to TMS (¹H and ¹³C) and trifluoroacetic acid (¹⁹F) (δ = -75.39), the solvent peak of CDCl₃ defined at δ = 7.26 ppm (¹H NMR) and δ = 77.16 (¹³C NMR), DMSO-d₆ defined at δ = 2.5 ppm (¹H NMR) and δ = 39.70 (¹³C NMR). Coupling constants are quoted in Hz (J). ¹H NMR splitting patterns were designated as singlet (s), doublet (d), triplet (t), quartet (q), (td) triplet of doublets and multiplet (m). Splitting patterns that could not be interpreted or easily visualized were designated as multiplet (m) or broad (br). Melting points were measured on solids after chromatography using a 1101D-MEL-TEMP melting point apparatus.

2. Synthesis of the Starting Materials

But-3-ynoic acid (2a).



To a round bottom flask equipped with a magnetic stirrer was added 65% HNO₃ (0.66 mmol, 0.045 mL), Na₂Cr₂O₇*2H₂O (0.13 mmol, 39 mg), NaIO₄ (29.06 mmol, 6.216 g) and water (11.9 mL) at 0 °C and stirred for 15 min. Subsequently, a cooled solution (0 °C) of 3-butyn-1-ol **4** (13.21 mmol, 1.0 mL) in water (11.9 mL) was added in one portion. The reaction mixture was vigorously stirred for 24 hours at r.t. The organic phase was separated from the aqueous slurry, which was subsequently extracted with diethyl ether (3 x 20mL), the organic phases was combined, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to afford but-3-ynoic acid (**2a**) as a white solid (0.90 g, 81%).¹ ¹H NMR (**400 MHz**,

CDCI₃) **5**: 3.38 (d, J = 2.8 Hz, 2H), 2.24 (t, J = 2.7 Hz, 1H). ¹³**C NMR (100 MHz, CDCI**₃) **5**: 173.6, 74.8, 72.5, 25.6, **FT-IR (neat)** υ (**cm**⁻¹): 3278, 2912, 2121, 1728, 1685, 1423, 1323, 1230, 871, 655, 489. **HRMS (ESI)**: Calc. For C₈H₉O₄ [2M+H]⁺ 169.0501; found: 169.0496. **Mp =** 76-79°C.

N-(3,5-dimethoxyphenyl)but-3-ynamide (2c).



To a stirred solution of but-3-ynoic acid **2a** (3.15 mmol, 0.26 g), 3,5-dimethoxyaniline, (3.0 mmol, 0.46 g) in DCM (13.6 mL) at 0 °C; was added *N*,*N'*-Dicyclohexylcarbodiimide (3.15 mmol, 0.65 g), the mixture was stirred and left under stirring until reaching r.t. gradually and then stirred for 16h at rt. The reaction mixture was refrigerated 1 hour, then filtered cold and the filtrate was dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by flash chromatography (CHX-DCM 50:50) to afford *N*-(3,5-dimethoxyphenyl) but-3-ynamide (**2c**) as a white solid (0,39 g, 60 %).² ¹H NMR (**400 MHz, CDCI**₃) **5**: 8.13 (s, 1H), 6.76 (d, *J* = 2.2 Hz, 2H), 6.27 (s, 1H), 3.79 (s, 6H), 3.37 (d, *J* = 2.7 Hz, 2H), 2.51 (t, *J* = 2.7 Hz, 1H). ¹³C NMR (**100 MHz, CDCI**₃) **5**: 164.1, 161.1, 138.9, 98.2, 97.2, 75.3, 55.5, 28.6. **FT-IR (neat)** υ (cm⁻¹): 3263, 3093, 2997, 2839, 1658, 1597, 1450, 1276, 1203, 817, 802, 678. HRMS (**ESI**): Calc. for C₁₂H₁₄NO₃ [M+H]⁺, 220.0974; found, 220.0978. **M.p:** 153-156°C.

2-phenylacetic acid (15).



To a round bottom flask equipped with a magnetic stirrer was added potassium cyanide (59.95 mmol, 2.94 g), tetrabutyl ammonium bromide (1.44 mmol, 0.532 g) in a mixture DCM-Water 8:2 (100 mL) followed by benzyl bromide **23** (40.4 mmol, 6.912 g) and the mixture was stirred vigorously for 48 hours. Stirring was stopped and the organic phase was syringed out, and the aqueous layer was extracted with DMC (4 x 30 mL) and the organic phases was combined, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by flash chromatography (CHX – 100%) to afford benzonitrile as a

yellow oil. To a round bottom flask equipped with a magnetic stirrer was added benzonitrile (4.329 g, 37.0 mmol), NaOH (4.863 g, 121.6 mmol) and water (45.2 mL). The mixture was refluxed at 92 °C for 15 hours. After cooling in an ice bath, concentrated HCl was added to give a white solid, which was filtered, and the aqueous layer was extracted with DCM (4 x 30 mL) and combined with the solid. The mixture was concentrated in vacuo to dryness to afford phenylacetic acid (**15**) as a white solid (4.570 g, 83.1 %).⁴ ¹H NMR (400 MHz, CDCl₃) **5**: 7.40 – 7.21 (m, 5H), 3.64 (s, 2H). ¹³C NMR (100 MHz, CDCl₃) **5**: 178.2, 133.4, 129.5, 128.8, 127.5, 41.2. FT-IR (neat) υ (cm⁻¹): 3032, 2735, 1689, 1604, 1408, 1338, 1226, 1072, 891, 752, 698. MS (EI): 136 [M+] (30), 91 (100), 65 (35), 51 (10). Mp= 74-77°C.¹H NMR and 1³C NMR spectra correspond to those reported previously.

3-fluoro-4-nitrophenol (18).



THF (4.0 mL) was added to ferric nitrate nonahydrate (2.23 mmol, 0.91 g). The mixture was stirred vigorously for 10 min at room temperature, then 3-Fluorophenol **24** (4.5 mmol, 0.5 g) was dissolved in THF, added to the nitrate mixture and the whole new mixture heated to 42 °C. The reaction was stirred overnight at this temperature, then filtered to remove the inorganic salts. The crude was concentrated under reduced pressure and purified by flash chromatography (CHX-DCM 50:50) to afford a mixture of 3-fluoro-4-nitrophenol (**18**) as a yellow solid (0.31 g, 43%).⁵ ¹H NMR (**400 MHz**, **DMSO-d**₆) δ = 11.50 (s, IH), 8.07 (t, *J* = 9.2 Hz, 1H), 7.23 – 6.40 (m, 2H).¹³C NMR (**101 MHz**, **DMSO-d**₆) = δ 164.9 (d, *J* = 12.4 Hz), 157.0 (d, *J* = 261.5 Hz), 128.8 (d, *J* = 6.6 Hz), 128.4 (d, *J* = 1.5 Hz), 112.3 (d, *J* = 2.6 Hz), 104.4 (d, *J* = 22.7 Hz). ¹⁹F-NMR (**374 MHz**, **DMSO-d**₆) δ : -115.1. FT-IR (neat) υ (cm⁻¹): 3228, 3113, 1627, 1600, 1473, 1323, 1276, 1153, 1049, 906, 821, 655. MS (EI) = 157 [M⁺] (60), 57 (100), 127 (70), 83 (80), 99 (40), 51 (40). M.p: 84-88°C.

4-amino-3-fluorophenol (19).



3-Fluoro-4-nitrophenol **18** (0.64 mmol, 0.1 g) in methanol (6.9 mL) was hydrogenated over 10% palladium on carbon (18 mg) at atmospheric pressure overnight. The catalyst was removed by filtration over celite. The crude was concentrated under reduced pressure and purified by flash chromatography (DCM-100%) to afford 4-amino-3-fluorophenol (**19**) as a yellow solid (0.074 g, 91%).⁶ ¹H NMR (400 MHz, DMSO-d₆) δ 8.77 (s, 1H), 6.67 – 6.53 (m, 1H), 6.42 (dd, *J* = 12.9, 2.4 Hz, 1H), 6.33 (d, *J* = 10.4 Hz, 1H), 4.36 (s, 2H).¹³C NMR (**101** MHz, DMSO-d₆) δ = 151.1 (d, *J* = 236.9 Hz), 148.7 (d, *J* = 9.8 Hz), 128.1 (d, *J* = 13.2 Hz), 117.3 (d, *J* = 5.9 Hz), 111.2 (d, *J* = 3.1 Hz), 103.0 (d, *J* = 21.0 Hz). ¹⁹F-NMR (**374** MHz, DMSO-d₆) δ : -130.7, FT-IR (neat) v(cm⁻¹): 3383, 3294, 2943, 2804, 2615, 1859, 1612, 1508, 1361, 1296, 1138, 902, 767. MS (ESI): 127 [M⁺] (100), 98 (90), 52 (60). M.p: 140-144°C.

2-amino-5-fluorophenol (20).



5-Fluoro-2-nitrophenol **25** (0.64 mmol, 0.1 g) in methanol (6.9 mL) was hydrogenated over 10% palladium on carbon (18 mg) at atmospheric pressure overnight. The catalyst was removed by filtration over celite. The crude was concentrated under reduced pressure and purified by flash chromatography (DCM-100%) to afford 2-amino-5-fluorophenol (**20**) as a grey solid (0.78 g, 96%).⁶ ¹H NMR (400 MHz, DMSO-d₆) δ: 9.41 (bs, 1H), 6.53 (dd, *J* = 8.6, 6.1 Hz, 1H), 6.46 (dd, *J* = 10.3, 2.8 Hz, 1H), 6.35 (td, *J* = 8.7, 2.9 Hz, 1H), 4.37 (s, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ: 154.4 (d, *J* = 231.0 Hz), 144.8 (d, *J* = 10.4 Hz), 133.2 (d, *J* = 2.3 Hz), 114.1 (d, *J* = 9.3 Hz), 104.9 (d, *J* = 21.3 Hz), 102.1 (d, *J* = 24.8 Hz). ¹⁹F-NMR (374 MHz, CDCl₃) δ: -77,0. FT-IR (neat) υ (cm⁻¹): 3379, 3313, 2889 ,2576, 2113, 1610, 1519, 144-147°C.



To a stirred suspension of 5-fluoro-2-nitrophenol **25** (6.4 mmol, 1.0 g) and K₂CO₃ (7.6 mmol, 1.06 g) in *N*,*N*-dimethylformamide (10 mL) was added benzylbromide **23** (7.0 mmol, 1.2 g) at room temperature. The reaction mixture was stirred at 60°C for 3 h. After the reaction was completed, the reaction mixture was diluted with water and extracted with EtOAc (3 x20 mL). The organic layer was washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure and purified by flash chromatography (Pentane-EtOAc 95:5) to afford 2-(benzyloxy)-4-fluoro-1-nitrobenzene (**21**) as a yellow solid (1.39 g, 88%).⁷ ¹**H NMR** (400 MHz, CDCl₃) δ : 7.97 (dd, *J* = 9.1, 5.9 Hz, 1H), 7.41 (ddd, *J* = 23.5, 17.1, 7.1 Hz, 5H), 6.83 (dd, *J* = 10.2, 2.6 Hz, 1H), 6.74 (ddd, *J* = 9.5, 7.1, 2.5 Hz, 1H), 5.22 (s, 2H). ¹³C NMR (100 MHz CDCl₃) δ : 165.6 (d, *J* = 256.1 Hz), 154.2 (d, *J* = 11.2 Hz), 136.4, 134.8,128.9, 128.5, 128.2 (d, *J* = 11.4 Hz), 127.0, 107.7 (d, *J* = 23.5 Hz), 102.9 (d, *J* = 26.8 Hz), 71.5. ¹⁹F-NMR (374 MHz, CDCl₃) δ : -112.0. FT-IR (neat) υ (cm⁻¹): 2939, 1745, 1621, 1512, 1411, 1264, 1014, 968, 837, 696. HRMS (ESI): Calc. for C₁₃H₁₀FNNaO₃ [M+Na]⁺, 270.0542; found, 270.0537. M.p: 46-50°C.





2-(benzyloxy)-4-fluoro-1-nitrobenzene **21** (5.1 mmol, 1.25 g) and Ethanol/H₂O (25 mL) were charged into a round flask. Iron powder (25.3 mmol, 1.41 g) and NH₄Cl (5.1 mmol, 0.27 g) were added to the mixture. The resulting mixture was refluxed for 1 h and then allowed to cool to room temperature. The resulting suspension was filtrated with a pad of celite, and the filtrate was concentrated in vacuo. The resulting mixture was diluted with EtOAc and then H₂O was added. The aqueous phase was extracted with EtOAc (3x 10 mL), and the combined organic phase was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (Pentane-EtOAc 95:5) to afford 2-(benzyloxy)-4-fluoroaniline (**22**) as a brownish oil (0.97 g, 78%).⁸ ¹H NMR (400 MHz, CDCl₃) δ : 7.49 – 7.31 (m, 5H), 6.71 – 6.62 (m, 2H), 6.59 – 6.50 (m, 1H), 5.06 (s, 2H), 3.69 (s, 2H). ¹³C NMR (100 MHz CDCl₃) δ : δ 156.3 (d, *J* = 235.7 Hz), 146.9 (d, *J* = 9.5 Hz), 136.9, 132.5 (d, *J* = 2.7 Hz), 128.8, 128.3, 127.7, 115.0 (d, *J* = 9.2 Hz), 107.0 (d, *J* = 21.9 Hz), 100.6 (d, *J* = 26.7 Hz), 70.7.¹⁹F-NMR (**374 MHz, CDCl₃)** δ : -123,5. FT-IR (neat) v(cm⁻¹): 3456, 3367, 2939, 1747, 1612, 1508,

1435, 1284, 1014, 960, 833, 698. **HRMS (ESI):** Calc. for C₁₃H₁₃FNO [M+H]⁺, 218.0981; found, 218.0974.

3. Antifungal Activity.

3.1. Fungal strain

Fusarium oxysporum fungal strain was provided by the research group in Bacteriología Agrícola y Ambiental (BA&A) at the Universidad de Antioquia in Colombia. This strain was isolated from the diseased stems of *Cannabis sativa*. To establish a pure culture, the fungus was subcultured on potato dextrose agar (PDA) medium at room temperature. The identification of the fungus was carried out using taxonomic keys and by observing its macroscopic and microscopic characteristics. In addition, molecular identification was performed by Sanger sequencing of the region ITS1-5.8S- ITS2 and a small portion of the 28S rDNA (approximately 900 bp), with the primers PN3 (5'-CCGTTGGTGAACCAGCGGAGGGATC-3') and PN16 (5'- TCCCTTTCAACAATTTCACG-3')⁹.

3.2. Antifungal activity of Microscale Amended Medium (MSAM) Assay

The in vitro evaluation was performed by measuring the mycelial growth halo of the phytopathogen Fusarium oxysporum using a micro-scale amended protocol using 12-well glass plates.¹⁰ PDA (Papa Dextrose Agar) medium was prepared at 1.5% and sterilized. The compounds were solubilized in a mixture of PDA (1.5%), DMSO (2%), and Tween 20 (5%), being evaluated at seven different concentrations (5-0.08 mg/mL), and the commercial fungicide epoxiconazole was used as a positive control. A volume of 180 µL of each concentration was placed in the excavated plates, and mycelium plugs of 1 mm in diameter were inoculated in the center of each well. Each test related to a randomized design with three replicates were performed for each treatment (Figure S1). The inoculated plates were placed in humid chambers for 72 hours at room temperature (average 17-19°C, relative humidity 64%). The mixture of PDA (1.5%), DMSO (2%), and Tween 20 (5%) was used as the control check group. Using the software ImageJ,¹¹ the mycelial growth areas were obtained, and the inhibition percentages were calculated using the following formula: % inhibition = (B-T) * (100/B), where B represents the diameter of mycelial growth for the control check, and T represents the diameter of mycelial growth of the treatments (Figure **S2**). The inhibition data (%) were used to build the corresponding dose-response curves to calculate the inhibitory concentration 50 (IC_{50} in mM) for each compound, using non-linear

regression in GraphPad Prism version 10.0.0 for Windows (GraphPad Software, San Diego, CA, USA).



Figure S1. Schematic representation of the randomly organized experimental design for the MSAM assays; B = control check (*F. oxysporum* without treatment), each number (1, 2, 3, 4, 5, 6 and 7) indicates the treatment concentration (in mg/mL) loaded into each well of the 12-well plate: 1 = 5 mg/mL; 2 = 2.5 mg/mL; 3 = 1.25 mg/mL; 4 = 0.62 mg/mL; 5 = 0.31 mg(mL; 6 = 0.16 mg/mL; 7 = 0.08 mg/mL. Created with Biorender.com.



Figure S2. In vitro antifungal activity of compounds against mycelial growth of Fusarium oxysporum.

4. Copies of NMR spectra

But-3-ynoic acid (2a)



N-(3,5-dimethoxyphenyl)but-3-ynamide (2c)



2-(benzofuran-2-yl) acetic acid (3a)



ОН













2-(benzofuran-2-yl)-N-(4-hydroxyphenyl) acetamide (10a)









10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 f1 (ppm)

2-(benzofuran-2-yl)-N-(4-fluoro-2-hydroxyphenyl) acetamide (10c)





0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 f1 (ppm)







2-(benzofuran-2-yl) propanoic acid (13a)



2-(benzofuran-2-yl) butanoic acid (13b)





2-(benzofuran-2-yl)-N-(2-fluoro-4-hydroxyphenyl) propanamide (14a).

f1 (ppm)

Ш

160 150





2-(benzofuran-2-yl)-N-(4-fluoro-2-hydroxyphenyl) propanamide (14b).











2-(benzofuran-2-yl)-N-(2-fluoro-4-hydroxyphenyl) butanamide (14c).





2-(benzofuran-2-yl)-N-(4-fluoro-2-hydroxyphenyl) butanamide (14d)





0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -2 f1 (ppm)













3-fluoro-4-nitrophenol (18)







4-amino-3-fluorophenol (19)







2-amino-5-fluorophenol (20)







2-(benzyloxy)-4-fluoro-1-nitrobenzene (21)







F O

0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -2(f1 (ppm)

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