Daylight LED promotes photochemical ring contraction of 2-amine-4H-pyran-3-carbonitriles with consequent loss of their antifungal activity

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Abstract

The initial objective of our work was to synthesize a series of 2-amino-4H-pyran-3-carbonitriles to be tested for their antifungal activities against economically relevant phytopathogenic fungi. Fourteen compounds were prepared in up to 94% yield and shown percentages of *Botrytis cinerea* inhibition above 70%. Despite the promising biological results, we observed that stock solutions prepared for biological tests showed color changing when kept for a few days on the laboratory bench, under room conditions, illuminated by common LED daylight tubes (4500–6000 k). This prompted us to investigate the possible photo-induced degradation of our compounds. FT-IR ATR experiments evidenced variations in the expected bands for functional of -amino-4H-pyran-3-carbonitriles stored under LED daylight. Following, HPLC–UV analysis showed reductions in the intensity of chromatographic peaks of 2-amino-4H-pyran-3-carbonitriles, and but not for solutions kept in the dark. A solution of (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile underwent 84.4% of conversion after 72 h of exposure to continuous LED daylight in a BOD chamber, and the reaction product was isolated in 36% yield and characterized as (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7***). Despite freshly prepared solutions of 2-amino-4H-pyran-3-carbonitriles produced antifungal activities, these solutions lost biological activity when left on the bench for a week. Besides, compound **7*** formed from photo-induced degradation of **7** also showed no antifungal activity. With this, we hope to bring two contributions: (1) production of cyclobutenes through photochemical reactions of 2-amino-4H-pyran-3-carbonitriles can be carried out through exposure to simple white LED daylight; (2) biological applications of such 2-amino-4H-pyran-3-carbonitriles may be impaired by their poor photostability.

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Graphic abstract

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1 Introduction

The pyran ring is chemically characterized as a six-membered heterocyclic ring containing oxygen as heteroatom and two carbon–carbon double bonds. Such structural moiety is found in several classes of secondary metabolites, including flavonoids, coumarins, iridoids, and lactones [1]. Many of such compounds have shown relevant medicinal properties and have inspired the synthesis and investigation of the biological activities of a variety of compounds presenting a pyran ring as a structural feature, including 2-amine-4H-pyran-3-carbonitriles. A series of works have shown that 2-amine-4H-pyran-3-carbonitriles exhibit a broad spectrum of biological effects of pharmaceutical interest, such as anti-cancer, anti-microbial, antihemolytic, and antioxidant activities [1–11].

Despite the wide number of studies on the medicinal properties of 2-amine-4H-pyran-3-carbonitriles, a few works have investigated biological activities of agricultural interest [12, 13]. As part of our efforts to search and develop novel agrochemical agents, we synthesized a series of 2-amine-4H-pyran-3-carbonitriles to be tested for their antifungal activities against agricultural relevant plant pathogens. Surprisingly, we observed that some of these compounds, prepared for the antifungal assays, showed color changes when kept for a few days on the bench. Taking into account

that 2-amine-4H-pyran-3-carbonitriles can undergo photochemical reactions [14–17], our find prompted us to prepare new solutions and keep them in the dark. Interestingly, no color change has occurred. In their studies, Armesto et al. [15–17] demonstrated that the pyran ring of 2-amine-4H-pyran-3-carbonitriles could undergo ring contraction or be transformed into 1,3-butadiene upon direct ultraviolet (UV) irradiation using Hg lamps.

However, to the best of our knowledge, the present work is the first report on the natural light-induced photochemical reaction of 2-amine-4H-pyran-3-carbonitriles. The photochemical conversion was investigated and proved using High-Performance Liquid Chromatography (HPLC–UV) and attenuated total reflectance-Fourier transform infrared (FT-IR ATR) experiments; chemical structure of the product from photochemical transformation was elucidated employing spectroscopic and spectrometric data.

2 Experimental

2.1 General chemistry

All reagents were purchased from commercially available suppliers and used as they were received. Solvents used in HPLC analysis were HPLC grade. Analytical thin-layer

chromatography (TLC) analysis was performed on aluminum-packed pre-coated silica-gel 60 G F₂₅₄ TLC plates (Macherey–Nagel, 0.25 mm, Düren, Germany), and revealed with potassium permanganate solution, vanillin, or ultraviolet (UV) light ($\lambda = 254$ and 366 nm). Preparative TLC was conducted on silica-gel 60 G F₂₅₄ (Merck, 1.0 mm) and revealed with UV light ($\lambda = 254$ nm). Melting points were obtained using a Microquímica MQAPF302 apparatus (Microquímica Equipamentos, Palhoça, Santa Catarina, Brazil). Electron impact-mass spectrometry (EI-MS) was performed in a Shimadzu GCMS-QP5050A equipment (Shimadzu Europe, Duisburg, Germany) by direct insertion, operating at 300 °C for 0.5 s, using EI mode (70 eV). FT-IR ATR was performed on a Varian 660-IR spectrophotometer (Varian, Palo Alto, CA, USA) equipped with a GladiATR Pike apparatus. ¹H and ¹³C Nuclear Magnetic Resonance (NMR) analyses were recorded on a Varian Mercury 500 instrument (Varian, Palo Alto, California, USA) at 500–300 MHz and 126–75 MHz, respectively, using chloroform or dimethylsulfoxide deuterated. High-resolution mass spectrometry (HRMS) was carried out on a high-resolution Q Exactive hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, San Jose, USA). The optimized Q Exactive parameters were as follows: electrospray source was operated in the positive mode, resolution 140,000 (FWHM at 200 m/z), spray voltage set at 4.5 kV, and capillary temperature 320 °C, S-Lens 60. The mass spectra were acquired and processed using Xcalibur software (Thermo Scientific). The UV/Vis spectroscopy analysis ($\lambda = 200$ –400 nm) was obtained in a double beam spectrophotometer (Micronal, model AJX-6100PC) using compound solutions at 250 $\mu\text{g mL}^{-1}$ in acetone. HPLC analysis was performed on a Shimadzu apparatus (Shimadzu Europe, Duisburg, Germany); using UV/Vis detector (SPD-10AV), automatic injector (SIL-10A); pump (LC-20AT), at the following operational conditions: silica-gel column (100 Å, 250 mm $\times$ 4.6 mm $\times$ 5 μm), flow rate of 1 mL min^{-1} ; sample injection volume 20.0 μL ; oven temperature 22 °C (CTO 10 ASVP); the pressure of 55–57 kgf cm^{-2} ranges; mixtures of dichloromethane/ethyl acetate (v/v) or dichloromethane/acetone (v/v) mobile phases and wavelength varying from 325 to 345 nm (UV _{λ_{max}} absorption length for each compound).

2.2 Synthesis of 2-amino-4H-pyran-3-carbonitriles 1–14

The synthesis of compound 1–12 was carried out according to the methodology described by Karimi-Jaberi and Pooladian [18]. To this, K₂CO₃ (0.05 mmol), α,α -bis(ylidene)cyclohexanones (1.0 mmol), malononitrile (1.1 mmol), and 10 mL of ethanol 96% (v/v) were added to a round-bottom flask, and the reaction was kept under stirring for 2–60 min under reflux. At the end of the reaction, the system was left

to cool to room temperature. The solid obtained was filtered, washed with ice-cold ethanol and hexane (10 mL each), kept in an oven at 55 °C until constant mass, and then subjected to chemical characterization. Compounds 13 and 14 were synthesized employing the same method but using a mixture of ethanol–water 7:3 (v/v) instead of ethanol as solvent.

2.2.1 (E)-2-amino-8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (1)

White solid; 84%; M.p.: 223–225 °C; MS (EI, 70 eV), m/z (%): 400 (100), 356 (81), 293 (62), 279 (41), 249 (28), 186 (26), 121 (60). IR (ATR) ν_{max} (cm^{-1}): 3470, 3367, 2944, 2915, 2842, 2192, 1673, 1636, 1506, 1249, 1024 and 833. ¹H NMR (500 MHz, CDCl₃) δ 7.24 (d, $J = 8.5$ Hz, 2H), 7.16 (d, $J = 8.3$ Hz, 2H), 6.90 (d, $J = 8.5$ Hz, 2H), 6.87 (d, $J = 8.3$ Hz, 2H), 6.81 (s, 1H), 4.51 (s, 2H), 3.91 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 2.71 (m, 1H), 2.56 (m, 1H), 1.96 (m, 2H), 1.62 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 158.8, 158.8, 158.4, 141.4, 135.1, 130.5, 129.6, 128.9, 127.9, 122.0, 120.1, 114.7, 114.1, 113.6, 60.8, 55.3, 55.2, 42.8, 27.4, 27.1, 22.3. HRMS (ESI) m/z , calcd. for C₂₅H₂₄N₂O₃ [M + H]⁺: 400.17869, found: 401.18381. UV _{λ_{max}} (nm): 211.0 and 330.0.

2.2.2 (E)-2-amino-8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (2)

Yellow solid; 66%; M.p.: 209–211 °C; MS (EI, 70 eV), m/z (%): 410 (21), 409 (16), 408 (35), 365 (37), 297 (100), 283 (32), 139 (26), 125 (34), 115 (26). IR (ATR) ν_{max} (cm^{-1}): 3448, 3324, 3214, 2915, 2842, 2199, 1673, 1637, 1593, 1410 and 827. ¹H NMR (500 MHz, CDCl₃) δ 7.49–7.18 (m, 8H), 6.81 (s, 1H), 4.56 (s, 2H), 3.95 (s, 1H), 2.67 (m, 1H), 2.54 (m, 1H), 1.86 (m, 2H), 1.62 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 158.8, 141.5, 141.3, 135.3, 133.2, 132.7, 130.5, 129.8, 129.2, 129.0, 128.4, 121.7, 119.6, 115.2, 60.2, 43.1, 27.4, 27.0, 22.1. HRMS (ESI) m/z , calcd. for C₂₃H₁₈Cl₂N₂O [M + H]⁺: 408.07962, found: 409.06720. UV _{λ_{max}} (nm): 211.0 and 329.0.

2.2.3 (E)-2-amino-8-(4-bromobenzylidene)-4-(4-bromophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (3)

White solid; 88%; M.p.: 210–212 °C; MS (EI, 70 eV), m/z (%): 498 (40), 455 (45), 374 (18), 341 (100), 327 (32), 195 (30), 128 (35). IR (ATR) ν_{max} (cm^{-1}): 3448, 3316, 2943, 2857, 2192, 1666, 1601, 827 and 512. ¹H NMR (500 MHz, CDCl₃) δ 7.47 (m, 4H), 7.14 (m, 4H), 6.79 (s, 1H), 4.55 (s, 1H), 3.94 (s, 1H), 2.67 (m, 1H), 2.53 (m, 1H), 2.10–1.81 (m,

2H), 1.62 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 158.8, 141.8, 141.5, 135.8, 132.0, 131.4, 130.8, 129.9, 129.6, 121.8, 121.4, 120.8, 119.6, 115.1, 60.1, 43.1, 27.4, 27.0, 22.1. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{Br}_2\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 495.97859, found: 496.96621. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.0 and 329.5.

2.2.4 (E)-2-amino-8-(2-(trifluoromethyl)benzylidene)-4-(2-(trifluoromethyl)phenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (4)

White solid; 83%. M.p.: 212–214 °C; MS (IE, 70 eV), m/z (%): 476 (28), 433 (10), 407 (23), 331 (100), 317 (29), 131 (131). IR (ATR) ν_{max} (cm^{-1}): 3499, 3375, 2951, 2183, 1673, 1637, 1593, 1097 and 768. ^1H NMR (500 MHz, CHCl_3): δ 7.69 (d, $J = 7.8$ Hz, 1H), 7.64 (d, $J = 7.8$ Hz, 1H), 7.59 (t, $J = 7.7$ Hz, 1H), 7.55–7.48 (m, 2H), 7.36 (q, $J = 7.4$ Hz, 2H), 7.30 (d, $J = 7.7$ Hz, 1H), 7.0 (s, 1H), 4.59 (s, 2H), 4.51 (s, 1H), 2.53–2.26 (m, 2H), 2.42 (m, 1H), 2.33 (m, 1H), 2.04 (m, 1H), 1.85–1.71 (m, 1H), 1.67–1.46 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 159.0, 142.8, 141.5, 135.8, 132.8, 131.3, 131.2, 130.9, 130.7, 128.8, 128.6, 128.4, 127.3, 127.0, 126.0, 125.9, 125.9, 125.8, 125.3, 125.3, 123.1, 123.1, 119.7, 119.2, 115.9, 60.5, 38.0, 27.0, 27.0, 22.1. HRMS (ESI) m/z , calcd. for $\text{C}_{25}\text{H}_{18}\text{F}_6\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 476.13233, found: 477.13815. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.0 and 326.0.

2.2.5 (E)-2-amino-8-(2-chloro-6-fluorobenzylidene)-4-(2-chloro-6-fluorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (5)

White solid; 90%; M.p.: 233–234 °C; MS (IE, 70 eV), m/z (%): 445 (14), 444 (27), 409 (89), 315 (100), 190 (21), 143 (50). IR (ATR) ν_{max} (cm^{-1}): 3455, 3338, 3185, 2951, 2834, 2184, 1673, 1637, 1601, 1401, 1403, 1133 and 779. ^1H NMR (300 MHz, CDCl_3): δ 7.25–7.13 (m, 4H), 7.05–6.92 (m, 2H), 6.56 (s, 1H), 4.89 (s, 1H), 4.59 (s, 2H), 2.25 (ddd, $J = 9.5, 4.6, 2.2$ Hz, 2H), 2.14–1.82 (m, 2H), 1.74–1.61 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3): δ 161.5, 160.1, 158.2, 135.1, 135.1, 133.5, 133.5, 129.3, 129.2, 128.9, 128.8, 125.1, 125.0, 124.4, 124.1, 119.5, 114.2, 113.9, 113.3, 56.8, 27.6, 27.5, 27.2, 21.8. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{16}\text{Cl}_2\text{F}_2\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 444.06077, found: 445.06830. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 211.0 and 325.0.

2.2.6 (E)-2-amino-8-benzylidene-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (6)

White solid; 94%; M.p.: 223–224 °C; MS (IE, 70 eV), m/z (%): 340 (40), 297 (37), 263 (100), 249 (20), 128 (12), 115 (21). IR (ATR) ν_{max} (cm^{-1}): 3426, 3331, 3024, 2943, 2915, 2827, 2184, 1666, 1410 and 695. ^1H NMR (300 MHz,

CDCl_3): δ 7.43–7.27 (m, 10H), 6.89 (s, 1H), 4.52 (s, 2H), 3.98 (s, 1H), 2.74 (m, 1H), 2.68–2.51 (m, 1H), 2.00 (m, 2H), 1.71–1.61 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 158.9, 142.8, 141.5, 137.0, 129.4, 129.2, 128.8, 128.2, 127.9, 127.4, 126.8, 122.6, 119.9, 115.3, 60.7, 43.6, 27.5, 27.1, 22.3. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 340.15756, found: 341.16354. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 211.0 and 327.0.

2.2.7 (E)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (7)

Orange solid; 76%; M.p.: 224–226 °C; MS (IE, 70 eV), m/z (%): 430 (19), 413 (15), 370 (28), 347 (100), 308 (76), 215 (22), 128 (37), 115 (42). IR (ATR) ν_{max} (cm^{-1}): 3477, 3353, 3076, 2922, 2834, 2184, 1666, 1629, 1506, 1337 and 840. ^1H NMR (400 MHz, CDCl_3): δ 8.24 (q, $J = 2.5$ Hz, 2H), 8.21 (q, $J = 2.6$ Hz, 2H), 7.47–7.40 (m, 4H), 6.94 (s, 1H), 4.67 (s, 2H), 4.14 (s, 1H), 2.81–2.52 (m, 2H), 2.15–1.83 (m, 2H), 1.68 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3): δ 159.0, 149.8, 147.4, 146.3, 143.5, 141.7, 132.3, 129.8, 128.8, 124.3, 123.6, 121.5, 119.1, 116.0, 59.2, 43.6, 27.5, 27.1, 22.0. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_5$ $[\text{M} + \text{H}]^+$: 430.12772, found: 431.13278. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 230.0 and 345.0.

2.2.8 (E)-2-amino-8-(4-fluorobenzylidene)-4-(4-fluorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (8)

White solid; 89%; M.p.: 217–219 °C; MS (IE, 70 eV), m/z (%): 376 (30), 333 (54), 281 (100), 267 (36), 174 (25), 133 (33), 109 (44). IR (ATR) ν_{max} (cm^{-1}): 3462, 3324, 2915, 2827, 2199, 1673, 1601, 1506, 1410, 1221 and 833. ^1H NMR (300 MHz, CDCl_3): δ 7.31–7.15 (m, 4H), 7.11–6.98 (m, 4H), 6.82 (s, 1H), 4.54 (s, 2H), 3.96 (s, 1H), 2.84–2.38 (m, 2H), 2.08–1.80 (m, 2H), 1.75–1.59 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3): δ 163.7, 163.3, 160.5, 160.0, 158.8, 141.4, 138.6, 138.6, 133.0, 132.9, 130.9, 130.8, 129.5, 129.4, 129.2, 129.2, 121.7, 119.7, 115.8, 115.5, 115.3, 115.0, 60.5, 42.9, 27.4, 27.0, 22.2. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{18}\text{F}_2\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: 376.13872, found: 377.14392. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 209.0 and 326.0.

2.2.9 (E)-2-amino-4-(pyridin-2-yl)-8-(pyridin-2-ylmethylene)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (9)

White solid; 79%; M.p.: 216–218 °C; MS (IE, 70 eV), m/z (%): 342 (54), 325 (20), 298 (33), 264 (46), 219 (100), 130 (34), 93 (46). IR (ATR) ν_{max} (cm^{-1}): 3470, 3353, 3082, 2908, 2834, 2177, 1666, 1579, 1410, 1133 and 746. ^1H NMR (300 MHz, CDCl_3): δ 8.59 (dddd, $J = 7.7, 4.8, 1.9, 0.9$ Hz, 2H), 7.65 (m, 2H), 7.25 (m, 2H), 7.18 (ddd, $J = 7.6,$

4.8, 1.1 Hz, 1H), 7.09 (ddd, $J=7.6, 4.8, 1.1$ Hz, 1H), 6.88 (s, 1H), 4.70 (s, 2H), 4.22 (s, 1H), 3.12–2.95 (m, 1H), 2.81 (m, 1H), 2.11 (dt, $J=17.2, 6.2$ Hz, 1H), 1.95 (dt, $J=17.2, 6.2$ Hz, 1H), 1.65 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 161.7, 159.6, 156.3, 149.6, 149.3, 141.7, 137.0, 135.9, 133.0, 124.8, 122.5, 122.3, 121.8, 121.2, 119.7, 116.4, 58.7, 46.0, 27.4, 27.0, 22.0. HRMS (ESI) m/z , calcd. for $\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}$ $[\text{M}+\text{H}]^+$: 342.14806, found: 343.15344. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.0 and 333.5.

2.2.10 (E)-2-amino-8-(4-ethylbenzylidene)-4-(4-ethylphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (10)

White-beige solid; 85%; M.p.: 144–146 °C; MS (IE, 70 eV), m/z (%): 396 (85), 367 (67), 352 (68), 291 (100), 277 (42), 247 (16), 128 (18), 119 (23). IR (ATR) ν_{max} (cm^{-1}): 3462, 3326, 2966, 2930, 2857, 2199, 1673, 1601, 1410 and 867. ^1H NMR (500 MHz, CDCl_3) δ 7.28–7.12 (m, 8H), 6.85 (s, 1H), 4.51 (s, 2H), 3.93 (s, 1H), 2.84–2.54 (m, 6H), 1.99 (m, 2H), 1.62 (m, 2H), 1.25 (m, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 158.9, 143.2, 142.9, 141.5, 140.1, 134.4, 129.2, 128.8, 128.2, 127.8, 127.7, 122.4, 120.1, 115.1, 60.7, 43.2, 28.6, 28.4, 27.5, 27.1, 22.3, 15.4, 15.4. HRMS (ESI) m/z , calcd. for $\text{C}_{27}\text{H}_{28}\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$: 396.22016, found: 397.22577. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.0 and 327.0.

2.2.11 (E)-2-amino-8-(3,4-dimethoxybenzylidene)-4-(3,4-dimethoxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (11)

White-beige solid; 75%; M.p.: 115–117 °C; MS (IE, 70 eV), m/z (%): 460 (100), 416 (45), 323 (60), 279 (22), 151 (59). IR (ATR) ν_{max} (cm^{-1}): 3608, 3382, 2930, 2834, 1673, 1601, 1506, 1140 and 1024; ^1H NMR (300 MHz, CDCl_3) δ 6.88 (m, 2H), 6.82 (m, 4H), 6.73 (d, $J=1.9$ Hz, 1H), 4.52 (s, 2H), 3.92 (s, 1H), 3.89 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 2.74 (m, 1H), 2.61 (m, 1H), 1.98 (t, $J=6.1$ Hz, 2H), 1.64 (m, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 158.7, 149.2, 148.5, 148.3, 148.0, 141.4, 135.5, 129.9, 128.2, 122.3, 121.9, 120.1, 120.0, 114.8, 112.6, 111.3, 110.9, 110.9, 60.7, 56.0, 55.9, 55.8, 43.2, 27.4, 27.2, 22.3. HRMS (ESI) m/z , calcd. for $\text{C}_{27}\text{H}_{28}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$: 460.19982, found: 461.20401. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.5 and 335.0.

2.2.12 (E)-2-amino-4-(furan-2-yl)-8-(furan-2-ylmethylene)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (12)

White solid; 57%; M.p.: 200–201 °C; MS (IE, 70 eV), m/z (%): 320 (100), 303 (17), 276 (58), 258 (30), 246 (28), 81 (29). IR (ATR) ν_{max} (cm^{-1}): 3448, 3309, 2944, 2842, 2184, 1673, 1607, 1410, 1155 and 732. ^1H NMR (500 MHz, CDCl_3) δ 7.43 (d, $J=1.8$ Hz, 1H), 7.35 (dd, $J=1.8, 0.8$ Hz,

1H), 6.62 (s, 1H), 6.44 (dd, $J=3.4, 1.8$ Hz, 1H), 6.35 (d, $J=3.4$ Hz, 1H), 6.32 (dd, $J=3.2, 1.8$ Hz, 1H), 6.19 (dt, $J=3.2, 0.8$ Hz, 1H), 4.57 (s, 2H), 4.13 (s, 1H), 2.88 (m, 1H), 2.69 (m, 1H), 2.16–2.03 (m, 2H), 1.79–1.66 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 159.6, 154.5, 152.9, 142.3, 142.1, 141.8, 126.9, 119.6, 113.2, 111.6, 110.7, 110.7, 110.3, 107.0, 57.5, 37.0, 27.2, 27.0, 21.7. HRMS (ESI) m/z , calcd. for $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 320.11609, found: 321.12203. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.5 and 331.5.

2.2.13 (E)-2-amino-8-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (13)

White-beige solid; 56%. M.p.: 238–240 °C; MS (IE, 70 eV), m/z (%): 372 (100), 328 (97), 279 (96), 265 (48), 234 (23), 172 (31), 107 (76). IR (ATR) ν_{max} (cm^{-1}): 3448, 3338, 3163, 2951, 2915, 2199, 1673, 1637, 1236, 1133 and 833. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) 9.52 (s, 1H), 9.30 (s, 1H), 7.19–7.08 (m, 2H), 7.01–6.94 (m, 2H), 6.83 (s, 1H), 6.80–6.68 (m, 4H), 6.64 (s, 2H), 3.81 (s, 1H), 2.71–2.55 (m, 1H), 2.49–2.38 (m, 1H), 2.04–1.65 (m, 2H), 1.52 (m, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 159.7, 156.3, 156.3, 140.5, 134.4, 130.4, 128.5, 127.6, 126.8, 121.9, 120.8, 115.3, 115.2, 114.6, 56.3, 42.4, 26.7, 26.5, 21.9. HRMS (ESI) m/z , calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$: 372.14739, found: 373.15335. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 210.5 and 329.5.

2.2.14 (E)-2-amino-8-(4-hydroxy-3-methoxybenzylidene)-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (14)

White-beige solid; 59%; M.p.: 175–177 °C; MS (IE, 70 eV), m/z (%): 432 (100), 388 (49), 366 (28), 309 (88), 232 (32), 200 (26) e 137 (59). IR (ATR) ν_{max} (cm^{-1}): 3616, 3426, 3331, 2937, 2937, 2915, 2192, 1666, 1637, 1506, 1418, 1257, 1140, 1024 and 739. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) 9.10 (s, 1H), 8.87 (s, 1H), 6.85 (s, 2H), 6.76 (m, 2H), 6.73–6.70 (m, 2H), 6.65 (s, 2H), 6.58 (dd, $J=8.1, 2.0$ Hz, 1H), 3.84 (s, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 2.78–2.59 (m, 1H), 2.50 (m, 1H), 2.08–1.95 (m, 1H), 1.89–1.72 (m, 1H), 1.53 (m, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ 159.7, 147.4, 147.2, 145.7, 145.5, 140.5, 135.0, 128.1, 127.0, 122.2, 122.1, 120.8, 119.9, 115.6, 115.3, 114.6, 113.3, 111.7, 56.2, 55.6, 55.5, 42.7, 26.7, 26.6, 22.0. HRMS (ESI) m/z , calcd. for $\text{C}_{25}\text{H}_{24}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$: 432.16852, found: 431.15805. $\text{UV}_{\lambda_{\text{max}}}$ (nm): 209.5 e 334.0.

2.3 Study on the photochemical conversion of 2-amino-4H-pyran-3-carbonitriles

First, solutions of the 2-amino-4H-pyran-3-carbonitrile at 3 mg mL^{-1} were prepared in dichloromethane (**1–12**) or

acetone (**13–14**) and kept resting at the bench under the room, uncontrolled conditions, illuminated by an LED daylight (4500–6000 K, 09 W, DSW Electric), about 8 h a day. All solutions were analyzed by HPLC–UV technique immediately, 7 and 14 days after preparation. Percentages of degradation of the compounds (%) were determined using Eq. (1)

$$\% \text{ Degradation} = \left(1 - \frac{AL}{A_{\text{control}}} \right) \times 100\%, \quad (1)$$

where AL, peak area after exposure to room conditions and A_{control} , peak area immediately after preparation.

Compounds **1**, **7**, and **12**, which showed higher conversion percentages, were selected for further studies. Two sets of solutions of these compounds at 1 mg mL^{−1} were then prepared in dichloromethane: the first set was kept under controlled light conditions in a BOD incubator, irradiated with daylight (LED daylight 5000 K, 20 W—GE) at 25 °C for 24, 48, and 72 h. For comparison, the second set of each solution was kept in the dark for 72 h at 25 °C. After this time, the samples were analyzed by HPLC–UV (as described above) and the percentage of conversion was calculated using Eq. 1.

2.4 Chemical characterization of compound (7*) formed from photochemical reaction of (7)

A solution of 2-amino-4*H*-pyran-3-carbonitrile (**7**) at 1 mg mL^{−1} in dichloromethane was kept in a BOD incubator under daylight for 24 h. After this, the solvent was evaporated under reduced pressure afforded a yellow solid subjected to preparative TLC eluted with hexane/ethyl acetate 3:7 (v/v). The product was scraped from the plate and recovered in ethyl acetate. Evaporation of the solvent under vacuum afforded a yellow solid that was identified as compound (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl) bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide.

2.4.1 (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl) bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (7*)

Yellow solid; 36%; Mp.: 215–217 °C; MS (EI, 70 eV), *m/z* (%): 430 (6), 387 (6), 347 (100), 317 (48), 308 (29), 215 (28), 115 (54), 66 (54); IR (ATR) ν_{max} (cm^{−1}): 3458, 3318, 2930, 2849, 2162, 1729, 1674, 1637, 1586, 1505, 1345, 1126 and 863; ¹H NMR (300 MHz, CDCl₃) δ 8.20 (t, *J*=9.0 Hz, 4H), 7.39 (t, *J*=8.7 Hz, 4H), 6.46 (s, 1H), 4.07 (s, 1H), 3.95 (s, 2H), 2.51 (m, 2H), 2.0 (m, 2H), 1.82 (m, 2H). ¹³C NMR (75 MHz, CDCl₃) δ 158.4, 149.7, 147.4, 146.3, 145.6, 141.6, 131.5, 129.8, 128.7, 124.5, 124.3, 122.6, 118.8, 117.1, 58.6, 43.2, 34.0, 27.8, 22.5. HRMS (ESI) *m/z*, calcd. for C₂₃H₁₈N₄O₅ [M+H]⁺: 430.12772, found: 431.13507.

2.5 Antifungal assays

Botrytis cinerea and *R. solani* isolates were obtained from the culture collection of the Universidade Federal de Viçosa—Coleção Octávio de Almeida Drummond (COAD2292 and COAD2390, respectively). The isolates were recovered by aseptic transfer of fragments of filter paper impregnated with fungal structures kept in silica-gel vials. These were seeded in plates containing potato dextrose agar (PDA). The fungi were maintained in a controlled temperature room at 25 °C under alternating 12-h daily light (light provided by two white and one near-UV lamps placed 35 cm above the plates)/12-h dark regime until inoculum harvest for experiments.

In vitro evaluations of the antifungal properties of each compound (**1–14** and **7***) against *B. cinerea* and *R. solani* were conducted by assessing the inhibition of radial growth of mycelium, according to the methodology described by Cimmino et al. [19]. Mycelial plugs (6 mm diameter) were excised from the margin of colonies growing in PDA plates and transferred to fresh PDA plates. Then, 200 nmol of each compound dissolved in 20 μ L of acetone was deposited on top of the mycelial plug surface (200 nmol per mycelial plug). Acetone was left to evaporate, and the plates were kept at 25 °C under a light regime as described above. Negative and positive controls consisted, respectively, of acetone and Captan SC (captana; p.a. 48.0%; ADAMA, 200 nmol plug^{−1}). The experiments were carried out in four replicates.

2.6 Statistical analysis

The analysis of variance and the Scott–Knott test was used to compare the means of *R. solani* and *B. cinerea* mycelial growth inhibition. These analyses were performed using Rstudio®. Paired two-tailed t test was used to compare inhibitions (%) by each compound with the positive control using the software GraphPad Prism® 8.0. In all cases, *p*<0.05 was considered statistically significant.

3 Results and discussion

3.1 Síntese de 2-amino-4*H*-pirano-3-carbonitrilas

Fourteen 2-amino-4*H*-pyran-3-carbonitriles (**1–14**) were synthesized through a methodology proposed (Scheme 1) in a single step, using ethanol as solvent, which is produced from renewable sources and a low-cost and low toxicity catalyst, K₂CO₃. Additionally, there was no need for chromatographic separations, which implies the economy of solvent. All these conditions are in agreement with the principles of Green Chemistry [20]. The -amino-4*H*-pyran-3-carbonitriles were obtained in low reaction times (2–60 min) and

Scheme 1 Synthesis and chemical structures of compounds **1–14**. Compounds **1–9**: K_2CO_3 (0.05 equiv.), α,α -bis(arylidene) cycloalkanes (1.0 equiv.) and malononitrile (1.1 equiv.), ethanol, reflux, 2–60 min.

*Compounds **13** and **14** were produced using ethanol–water 7:3 (v/v) as solvent. Yields are represented in parentheses

yields varying from 29 to 94% (Table S1, Supplementary Information).

When the synthesis of compounds **13** and **14** was carried out in ethanol, very low amounts of product precipitated from the reaction medium, and the GC–MS analysis of the supernatant showed that the desired remained in the supernatant. Therefore, we found out that adding a small amount of water to the reaction medium and keeping the reaction for a few more hours substantially improved the formation of the precipitates. Then, both reactions were carried out in ethanol–water 7:3 (v/v) instead of pure ethanol, and the compounds **13** and **14** were obtained in 56 and 59% yield, respectively.

Spectroscopic and spectrometric techniques characterized the synthesized substances, and all obtained spectra are presented in Supplementary Information (Fig. S1–S89). The obtained mass spectra showed the molecular ion peaks for all synthesized compounds, and m/z are consistent with their corresponding molecular formulas. The IR spectra showed bands at 3426–3608 cm^{-1} and 3309–3382 cm^{-1} ranges, both corresponding to N–H stretching ($\nu_{\text{N-H}}$) and consistent with the presence of the amine group of the cyclohexanone ring. In addition, bands at 2177–2199 cm^{-1} ranges were evidenced, characteristics of $\text{C}\equiv\text{N}$ stretching ($\nu_{\text{C}\equiv\text{N}}$), confirming the reaction by the appearance of the nitrile group. In the ^1H -NMR spectra, the absorption signals corresponding to the hydrogen from 4*H*-pyran nucleus and amine group were observed as singlets at δ 3.91–4.89 ranges and δ 4.51–4.68 ranges, respectively [18]. For instance, the mass spectra for compound

7 exhibited the peak of the molecular ion at m/z 430 (Fig. S42, Supplementary Information). The fragments at m/z 413 and 308 are consistent with the losses of the amine and aryl groups, respectively. The peak base at m/z 347 is following the internal loss of the aromatic ring. The infrared (IR) spectrum of **7** (Fig. S43, Supplementary Information) showed bands at 3477 and 3353 cm^{-1} (stretch N–H), 3076 (stretch $\text{C}_{\text{sp}2}\text{--H}$), 2922 and 2834 (stretch $\text{C}_{\text{sp}3}\text{--H}$), 2184 cm^{-1} (stretch $\nu_{\text{C}\equiv\text{N}}$), and 1666 cm^{-1} (angular deformation N–H). The ^1H nuclear magnetic resonance (^1H NMR) spectrum of compound **7** (Fig. S44, Supplementary Information) showed a singlet at the δ 4.14, referring to the hydrogens of the 4*H*-pyran ring. A singlet at the δ 4.67 integrated to two hydrogens was attributed to the hydrogens of the amine group, and the singlet at the δ 6.94 corresponds to the vinyl hydrogens. The signals corresponding to the hydrogens in the cyclohexane ring are observed as multiples at the δ 2.81–2.52, δ 2.15–1.83, and δ 1.68. The aromatic hydrogens are referents to quartets at the δ 8.24 ($J=2.5$ Hz), and δ 8.21 ($J=2.6$ Hz), and multiples at the δ 7.47–7.40. In the ^{13}C NMR spectrum of compound **7** (Fig. S45, Supplementary Information) was observed a signal at the δ 121.5, characteristic of the nitrile group, and at the δ 123.6 and 128.8, referring to the vinyl carbons. The signals of the 4*H*-pyran ring were observed at the δ 159.0, 141.7, 116.0, 59.2, and 43.6. The signals at the δ 22.0, 27.1, and 27.4 were attributed to the carbons CH_2 from the cyclohexane ring. The signals observed at the δ 149.8, 147.4, 146.3, 143.5, 132.3, 129.8, 124.3, and 119.1 correspond to the aromatic ring nucleus carbons.

All these data agree with those previously reported for this compound [2, 21]. Finally, the experimental value of the exact mass is in agreement with the calculated value.

3.2 Study on the photochemical conversion of 2-amino-4*H*-pyran-3-carbonitriles

The work aimed to investigate antifungal activities of the 2-amino-4*H*-pyran-3-carbonitriles against some phytopathogenic fungi. However, we observed that solutions of the compounds in acetone, prepared to be used in the antifungal assays, underwent color changes when kept on the bench under natural light and LED daylight tube for about 8 h a day, for a few days. Thus, considering that 2-amine-4*H*-pyran-3-carbonitriles can undergo photochemical reactions upon direct UV irradiation using Hg lamps [14–17], our observations led us to consider the possible light-induced transformation of our compounds, even under white light irradiation.

Thus, at first, the solutions of all synthesized 2-amine-4*H*-pyran-3-carbonitriles were again prepared in dichloromethane (compounds **1–12**) or acetone (compounds **13–14**) and kept for 14 days on the laboratory bench, under room conditions, illuminated by an LED daylight tube. Again, almost all solutions underwent a color change, as depicted in Fig. 1.

These solutions were analyzed by HPLC–UV immediately after preparation and then at 7 and 14 days of exposure to white light. Spectrophotometric UV/Vis experiments previously selected the absorption waves used in the HPLC analysis for each compound and, together with the mobile phase used to elute each compound, are reported in the Supplementary Information (Table S3). All chromatograms are also presented (Fig. S90–S102). The HPLC–UV evidenced a reduction of the peak area corresponding to each compound from 0 to 14 days. Compound **7** showed the highest conversion, and the chromatograms obtained for this compound are presented as an example (Fig. 2). This chromatogram

Fig. 2 HPLC–UV ($\lambda = 345$ nm) chromatogram of compound **7** during 0, 7, and 14 of exposure to white light. Mobile phase: dichloromethane–ethyl acetate 95:5 (v/v)

clearly shows a decrease of compound **7** and the increase of the reaction product of **7**, marked as **7***.

The percentages of conversion were calculated based on the decrease in the peak intensity of each compound and varied from 5.0 to 41.4% after 14 days (Fig. 3). No clear relationship between the nature of the substituents at the pyran ring and the percentages of conversion was observed.

After HPLC–UV analysis, the solvents were evaporated, and the compounds were subjected to FT-IR ATR techniques to verify variations in the expected bands for functional groups, and spectra are shown in the Supplementary Information (Fig. S103–S116). In general, variations in the intensity of the specific bands of some functional groups were observed, mainly for the bands at 3477 and 3353 cm^{-1} , that correspond to N–H stretching ($\nu\text{N-H}$), at 2177 to 2199 cm^{-1} characteristics of C $\equiv$ N stretching ($\nu\text{C}\equiv\text{N}$), and at 1666 and

Fig. 1 Color of the solutions of 2-amino-4*H*-pyran-3-carbonitriles in dichloromethane (**1–12**) and acetone (**13–14**) immediately after preparation (0 day) and at 7 and 14 days after preparation

Fig. 3 Percentage of conversion (%) of the 2-amino-4*H*-pyran-3-carbonitriles (**1**–**14**) after 7 and 14 days of exposure to LED light

1629 cm^{-1} , that correspond to angular deformation of N–H and C=C stretching ($\nu\text{C}=\text{C}$), respectively.

Although no relationship between the type of substituent (furyl or aryl) attached to the pyran ring or the substitution pattern of the benzene ring and the percentage of conversion has been observed, compounds (**1**), (**7**), and (**12**) were selected to additional experiments. Compound (**1**) and (**7**) present an electron donor group and an electron-withdrawing group at the *para* position of the aromatic rings, respectively, and compound (**12**) that presents a furan-2-yl group instead of a benzene ring attached to the pyran ring. Two sets of solutions of these compounds were prepared: the first set exposed to continuous controlled white light in a BOD chamber, and the second set was stored in the dark. HPLC–UV analyzed aliquots of all these solutions at 24, 48, and 72 h. All solutions exposed to light underwent color change (Fig. S117, Supplementary Information), which was not observed for solutions stored in the dark. As expected, the results from HPLC analysis showed that the 2-amino-4*H*-pyran-3-carbonitriles exposed to continuous white light underwent some chemical reaction, with the percentage of conversion up to 84.4% after 72 h of exposure (Table 1). There was no variation in the peak area of these 2-amino-4*H*-pyran-3-carbonitriles when protected from light.

All these solutions were subjected again to FT-IR ATR analysis and the spectra are shown in the Supplementary Information (Fig. S121–S122). In general, the variations in the intensities of the characteristic bands of some functional groups were similar to those observed in the analysis of the samples kept on the bench for 14 days under natural and LED daylight tubes. In the FT-IR ATR spectrum of the solution of compound **7** (Fig. 4), it was possible to observe the reduction of the intensity and the widening of the bands at 3477 and 3353 cm^{-1} ranges, both corresponding to N–H

Table 1 Photosensitivity of 2-amino-4*H*-pyran-3-carbonitriles **1**, **7**, and **12** after 24, 48 and 72 h of exposure to controlled white light

Compound	R	Percentage of conversion (%)			
		^a Control (72 h)	24 h	48 h	72 h
1	4-MeO-C ₆ H ₄	–	22.5	53.1	68.5
7	4-NO ₂ -C ₆ H ₄	–	76.9	82.6	84.4
12	Furan-2-yl	–	46.7	69.3	76.2

^aSample sheltered from light for 72 h

stretching (stretch N–H), and the emergence of a band at 701 cm^{-1} , compatible with carbonyl C=O (stretch C=O). The appearance of strong bands at 732 and 702 cm^{-1} indicates a possible variation in the content of double bonds present in the 4*H*-pyran nucleus. Since the absorptions at 1000 to 600 cm^{-1} range are related to the angular deformation of the C=C bond, there was variation in the unsaturation content in the molecule, probably with the deconstruction of the 4*H*-pyran nucleus. The characteristic band of the nitrile group at 2199 cm^{-1} (stretch C≡N) also showed little variation, suggesting that this group remains in the degradation product. Altogether, these data agree with the possible formation of an amide group, which also occurs when 2-amino-4*H*-pyran-3-carbonitriles are irradiated with UV light using a Hg lamp [14–17].

Fig. 4 FT-IR (ATR) spectrum of compound **7** at 0, 24, 48, and 72 h of exposure to controlled white light in a BOD incubation

Because compound **7** showed the highest percentage of photo-induced transformation, as evidenced by HPLC–UV experiments, a solution of this compound was prepared in acetone and continuously exposed to LED daylight for 24 h, and then subjected to column chromatography. The product of the photochemical reaction of **7** was obtained as a yellow solid (compound **7***) in 36% yield, and its identity was proved based on spectroscopic and spectrometric data (all spectra are presented in Supplementary Information, Fig. S123–S136).

HRMS of **7*** indicated a molecular formula $C_{23}H_{24}N_4O_5$. In the EI-MS analysis (Fig. S123, Supplementary Information), the peak of the molecular ion was observed at m/z 430, which is compatible with a molecular formula from HRMS analysis. The FT-IR ATR spectrum for **7*** demonstrated bands at 3458 and 3318 cm^{-1} (νNH_2), 2162 cm^{-1} ($\nu\text{C}\equiv\text{N}$), and 1729 cm^{-1} ($\nu\text{C}=\text{O}$ of amide group) (Fig. S124, Supplementary Information). In addition, the EI-MS spectrum showed a fragment at m/z 387 compatible with the losses of an amide group by the molecular ion, in agreement with data from FT-IR ATR. In the ^1H NMR spectrum (Fig. S125, Supplementary Information) of **7***, the singlets at the δ 6.46, 4.07, and 3.95 were observed for the hydrogens H-7, H-8, and NH_2 . Multiplets integrated for two hydrogens each were also observed at the δ 2.51, 2.0, and 1.82, referring to the hydrogens H-4, H-2, and H-3, respectively, present in the cyclohexane ring. In the ^{13}C NMR spectrum (Fig. S126, Supplementary Information), 23 carbons were identified, and the signal corresponding to the carbonyl of the amide group at the δ 158.4 was observed. In DEPT-135 (Fig. S127, Supplementary Information), the presence of three CH_2 carbons and eight CH groups was showed, compatible with the structure of the compound **7***. The COSY ^1H – ^1H NMR spectrum (Fig. S128, Supplementary Information) allowed

us to confirm the coupling of hydrogens H-4 and H-2 with H-3. In the HSQC ^1H – ^{13}C NMR spectrum (Fig. S129–S131, Supplementary Information), the hydrogens at the δ 2.51, 2.0, and 1.82 correlated with the carbon signals at the δ 34.0, 27.8, and 22.5, corresponding to the C-4, C-3, and C-2 carbons, respectively. The hydrogen signals at the δ 6.46 and 4.07 were related to the carbon signals at the δ 124.5 and 43.2, referring to the C-7 and C-8, respectively. The HMBC ^1H – ^{13}C NMR spectrum (Fig. S131–S133, Supplementary Information) allowed us to assign all the signals of the hydrogens and the carbons present in the structure of the compound **7*** (Fig. 5 and Table S4, Supplementary Information). In the NOESY ^1H – ^1H NMR spectrum (Fig. S134 and S135, Supplementary Information), it was possible to observe the interaction between the hydrogens H-4 and H-2'' of the cyclohexane ring, confirming the proximity of these hydrogens, characteristic of the stereochemistry E (Fig. 5).

Interestingly, a series of dihydropyrano[2,3-*c*]chromenes, with structures closely related to the 2-amine-4*H*-pyran-3-carbonitriles, was synthesized in very good yields by reacting aromatic aldehydes, malononitrile, and 4-hydroxycoumarin under photochemical activation by visible light [22]. In the present work, we show that through irradiation with a simple LED daylight tube that the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles can undergo ring contraction to produce a cyclobutene ring. Ring contraction of 4*H*-pyran to cyclobutene has already been reported, but using more drastic reaction conditions through irradiating 2-amine-4*H*-pyran-3-carbonitriles with UV light using an apparatus Pyrex filter and 400 W medium-pressure Hg arc lamp [15–17] (Scheme 2). A mechanism to such reaction, involving an intramolecular single-electron transfer followed by transannular bond formation and ring-opening, has been proposed [15–17]. However, we report for the first time that

Fig. 5 HMBC ^1H – ^{13}C NMR and NOESY ^1H – ^1H NMR correlation for compound **7***

Scheme 2 Comparison between the reaction conditions applied for photochemical ring contraction of 2-amine-4*H*-pyran-3-carbonitriles ring using UV light (Armesto et al. [15–17]) and LED light (present work)

such ring contractions can undergo under common laboratory illuminating conditions.

Initially, the color changes observed for the compounds, which led us to suppose that photoreaction took place, occurred in solutions using acetone as a solvent. This could lead to the conclusion that the reaction is due to sensitization with acetone, which is well known as a photosensitizing solvent [23–25]. However, the subsequent experiments on the photoconversion of the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles also occurred using dichloromethane as a solvent for most compounds, including for compound **7**, for which the highest percentage of conversion was observed. Thus, we can conclude that the photo-induced contraction of the 4*H*-pyran ring is not due to the sensitizing effect of acetone but, in fact, to the poor photostability of these compounds, which may be a limiting factor to their uses for certain biological purposes.

3.3 Antifungal assays

The fourteen compounds synthesized here as well as the product of photochemical degradation (**7***) were evaluated for their ability to inhibit the mycelial growth of *R. solani* and *B. cinerea* (Table S2, Supplementary Information). None of the substances proves to inhibit the growth of *R. solani* at 200 nmol μg^{-1} (data not shown). Results for the compounds that had antifungal activities against *B. cinerea* are presented in Fig. 6. Most of 2-amine-4*H*-pyran-3-carbonitriles inhibited *B. cinerea*, including the substance **7** (Fig. 6, black bar). However, interestingly, **7*** lost the effect against this fungus compared to its precursor, suggesting that the synthesized compounds might decrease the propriety of

Fig. 6 Percentage inhibition (%) of *Botrytis cinerea* mycelial growth by 2-amine-4*H*-pyran-3-carbonitriles at 200 nmol μg^{-1} , relative to the negative control (acetone). The compound **7**, target of the study of photochemical degradation, is represented in black bar. Captan is a positive control. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.005$, * $p < 0.05$ according to t test

inhibited the *B. cinerea* when possibly undergoing photochemical degradation.

4 Conclusion

A series of 2-amine-4*H*-pyran-3-carbonitriles were synthesized and tested for antifungal activity against important phytopathogenic fungi. Fourteen compounds were prepared in up to 94% and shown percentages of *Botrytis cinerea* inhibition above 70%. Additionally, it was

found out that all these compounds' solutions showed some color changes when kept on the bench for a few days. From this observation, a series of HPLC and FT-IR analyses were carried out and showed that these 2-amine-4*H*-pyran-3-carbonitriles underwent photochemical reaction by exposure to common LED daylight. (*E*)-2-amino-8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (**7**) displayed the highest percentage of conversion, and the product from its photo-induced reaction was isolated and identified as (*E*)-7-cyano-5-(4-nitrobenzylidene)-8-(4-nitrophenyl)bicyclo[4.2.0]oct-1(6)-ene-7-carboxamide (**7***). With this, we demonstrated that the 4*H*-pyran ring of 2-amine-4*H*-pyran-3-carbonitriles can undergo ring contraction to produce butene upon irradiation with LED daylight. Ring contraction of 4*H*-pyran to the cyclobutene has already been reported when 2-amine-4*H*-pyran-3-carbonitriles are irradiated by UV light using a Hg lamp. However, to the best of our knowledge, the present work is the first report on such conversion being promoted by simple LED daylight under a standard laboratory environment. Finally, freshly prepared solutions of 2-amine-4*H*-pyran-3-carbonitriles showed moderate-to-good antifungal activity against *B. cinerea*, a phytopathogenic of several economically significant strawberries, grape, kiwifruit, tomato, roses, and many other vegetables, ornamental and fruit crops. Despite freshly prepared solution of compound (**7**) inhibited in $74 \pm 3.54\%$ the mycelial growth of *B. cinerea*, the cyclobutene (**7***), produced from the photo-induced reaction of (**7**), showed no antifungal activity. It implies that the 4*H*-pyran ring is a key structural moiety to the antifungal activity but also suggests that this compound might have a limited application in agriculture because of its very low photostability.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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