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Synthesis and antimicrobial screening of heterocycles derived from 3-[(4-benzyloxy)phenyl]-1-(3-chlorophenyl)prop-2-ene-1-one

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Starting from the title enone (whose single crystal X-ray structure is being reported herein), a small library of structurally diverse heterocycles has been assembled through ring closure reactions with the view to evaluate their antimicrobial activity. A multi-step sequence comprising the reaction of the mentioned chalcone analogue with hydrazine, the *N*-acylation of the resulting pyrazoline with bromoacetyl bromide and the replacement of the easily leaving halogen atom in the acylated pyrazoline with a 4-methylumbelliferone moiety afforded a pyrazoline–coumarin hybrid. Investigation of the reaction of this chalcone analogue with guanidine allowed the isolation of a 2-aminopyrimidine derivative, while an imidazolone was obtained from guanidine and the epoxide of the chalcone analogue. Cyclocondensation of the chalcone analogue with 2-aminobenzenethiol yielded the expected benzothiazepine derivative. Reaction of the chalcone analogue with malononitrile in the presence of sodium methoxide in methanol led to a mixture of two structurally related pyridines. The heterocycles obtained from the title chalcone analogue were devoid of antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* at a concentration of 100 mg/mL.

Keywords: chalcone, cyclocondensation, heterocycles, single crystal X-ray diffraction, antimicrobial

INTRODUCTION

1,3-Di(aryl)prop-2-en-1-ones and structurally similar compounds in whose structure the aryl moieties are heteroaromatic ring systems [1] are commonly known as chalcone analogues. Owing to the presence of the reactive α, β-enone segment in their structure, the chemistry of chalcone analogues is governed by 1,4-nucleophilic addition reactions (generally known as Michael additions) [2, 3] and by ring closure reactions [4, 5]. Chalcone and its analogues are versatile synthons in organic synthesis, as a single member of this class of substances can be converted into multiple types of acyclic, carbocyclic or heterocyclic compounds through the appropriate choice of reagent and reaction conditions, and generate in a facile manner small diversified libraries of chemical entities with identical substituents grafted onto structurally distinctive scaffolds. Although the antimicrobial activity of several chalcone analogues having a 4-(benzyloxy)phenyl moiety at C-3 of the prop-2-en-1-one fragment in their structure and of their derivatives has been reported in literature [6–10], the information is still scarce and limited to a small number of compounds. The present study reports the synthesis and structural

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characterisation of 3-[(4-benzyloxy)phenyl]-1-(3-chlorophenyl)prop-2-ene-1-one, a hitherto unreported chalcone analogue, along with its conversion into several structurally diverse compounds with heterocyclic scaffolds, whose antimicrobial activity has been subsequently examined.

EXPERIMENTAL

Materials and instrumentation

The reagents used in this study (3-chloroacetophenone **1**, 4-(benzyloxy)benzaldehyde **2**, hydrazine hydrate, bromoacetyl bromide, 4-methylumbelliferone, guanidine hydrochloride, 2-aminothiophenol, malononitrile, anh. K_2CO_3, KOH, 30% H_2O_2, NaOH, 36% HCl) were purchased from Merck–Sigma–Aldrich, and were used without prior purification. The solvents were obtained from Merck–Sigma–Aldrich or VWR International, and were used without prior purification. Melting points were taken on a Mel-Temp II apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400-MHz spectrometer. The signals owing to residual protons in the deuterated solvents were used as internal standards for the ^{1}H NMR spectra (δ = 7.26 ppm for $CDCl_3$ and δ = 2.51 ppm for DMSO-d_6). The chemical shifts for the carbon atoms are given relative to residual chloroform (δ = 77.1 ppm) or dimethyl sulfoxide (δ = 39.5 ppm) in the corresponding deuterated solvents. The assignment of the signals in the NMR spectra was based on additional 2D homo- and hetero-nuclear correlation experiments (H,H-COSY, H,C-HSQC and H,C-HMBC). NMR spectra of compounds **3**–**12** can be found in the Electronic Supplementary Information associated with this article. Elemental analysis was performed on a Vario EL III CHNS analyzer.

Single crystal X-ray diffraction measurements were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer with graphite-monochromated MoKα radiation. The single crystal was positioned at 40 mm from the detector and 459 frames were measured each for 5 s over 1° scan width. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction [11]. The structures were solved by Intrinsic Phasing using the Olex2 software [12] with the SHELXT structure solution program [13] and refined by full-matrix least-squares on F^2 with SHELXL-2015 [14] using an anisotropic model for non-hydrogen atoms. The H atoms attached to carbon were introduced in idealised positions (d_{CH} = 0.96 Å) using the riding model, while those attached to oxygen were localised from Fourier maps, and their positional parameters were refined according to the parameters of hydrogen bonds. The single crystal of compound **3** that was suitable for the X-ray diffraction experiment was selected from the large sample obtained after recrystallisation from ethyl acetate of the material isolated after the work-up of the reaction mixture as described in Experimental.

Synthesis of 3-[(4-benzyloxy)phenyl]-1-(3-chlorophenyl)prop-2-ene-1-one 3

A solution of 3-chloroacetophenone **1** (1.545 g, 10 mmol) and 4-(benzyloxy)benzaldehyde **2** (2.12 g, 10 mmol) in 96% ethanol (20 mL) was treated with 40% aq. NaOH (0.2 mL). The reaction mixture was stirred at room temperature for 1 h, and then the resulting suspension of the chalcone analogue was set aside overnight. The solid was filtered, washed sequentially with a mixture of hexanes-2-propanol (2 × 10 mL, 1:2 v/v) and hexanes (2 × 10 mL), and air-dried. The material was dissolved in the minimum volume of chloroform (approximately 50 mL) under efficient stirring, the resulting light suspension was filtered gravitationally, and then chloroform from the filtrate was removed under reduced pressure to give the crude chalcone analogue, which was recrystallised from ethyl acetate (20–25 mL) to afford bright yellow crystals (2.23 g, 64%), mp 145–146°C (ethyl acetate). ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 5.13 (s, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 7.31–7.48 (m, 7H), 7.54 (ddd, *J* = 0.8, 2.0 and 8.0 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.80 (d, *J* = 15.2 Hz, 1H), 7.88 (dt, *J* = 1.2 and 8.0 Hz, 1H), 7.97 (t, *J* = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 70.1, 115.4, 119.2, 126.5, 127.5, 127.6, 128.2, 128.5, 128.7, 129.9, 130.4, 132.5, 134.9, 136.3, 140.1, 145.5, 161.1, 189.1. Anal. calcd. for $C_{22}H_{17}ClO_2$, %: C, 75.75; H, 4.91. Found: C, 75.41; H, 4.82.

Synthesis of 5-[4-(benzyloxy)phenyl]-3-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazole 4

A suspension of chalcone analogue **3** (1.394 g, 4 mmol) in 2-propanol (20 mL) was treated with hydrazine hydrate (400 mg, 8 mmol), and then

the mixture was heated at reflux temperature for 4 h. Refrigeration of the reaction mixture overnight afforded a solid, which was filtered, washed sequentially with a mixture of hexanes-2-propanol (2 × 10 mL, 1:1 v/v) and with hexanes (2 × 10 mL), and then it was air-dried to give the title compound as a colourless solid (1.32 g, 85%), mp 72–73°C; ^{1}H NMR (DMSO-d_6, 400.1 MHz), δ (ppm): 2.84 (dd, J_{AM} = 16.4 Hz and J_{AX} = 10.8 Hz, 1H, C4-H_A), 3.40 (dd, J_{AM} = 16.4 Hz and J_{MX} = 10.8 Hz, 1H, C5-H_M), 4.83 (td, J_{AX} = J_{MX} = 10.4 Hz and $J_{NH\text{-}Hx}$ = 2.8 Hz, 1H, C5-H_X), 5.10 (s, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.31–7.48 (m, 7H), 7.57 (dd, *J* = 7.2 and 1.2 Hz, 1H), 7.63 (t, *J* = 1.6 Hz, 1H), 7.72 (d, *J* = 2.8 Hz, 1H, NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz), δ (ppm): 40.1, 63.3, 69.1, 114.7, 123.8, 124.7, 127.5, 127.6, 127.7 (2 carbon atoms), 128.4, 130.4, 133.3, 134.9, 135.5, 137.1, 147.0, 157.5.

Synthesis of 1-{5-[4-(benzyloxy)phenyl]-3-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl}-2-bromoethanone 5

To a stirred solution of *N*^{1}-unsubstituted pyrazoline **4** (1.16 g, 3.2 mmol) in chloroform (20 mL), anh. K_2CO_3 (1.104 g, 8 mmol) and a solution of bromoacetyl bromide (970 mg, 4.8 mmol) in chloroform (10 mL) were sequentially added. After the mixture had been stirred at room temperature overnight, the inorganics were filtered off, and then the solvent in the filtrate was removed under reduced pressure. The resulting dense oil was extracted with hot 2-propanol (10 mL), and the operation was repeated with the oil that separated upon cooling. Finally, the solid that resulted was recrystallised from 96% ethanol to afford a colourless solid (990 mg, 64%), mp 87–88°C; ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 3.18 (dd, J_{AX} = 4.6 Hz and J_{AM} = 17.8 Hz, 1H), 3.74 (dd, J_{MX} = 11.7 Hz and J_{AM} = 17.8 Hz, 1H), 4.30 (d, *J* = 10.4 Hz, 1H), 4.34 (d, *J* = 10.4 Hz, 1H), 5.03 (s, 2H), 5.56 (dd, J_{AX} = 4.6 Hz and J_{MX} = 11.7 *Hz*, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.28–7.47 (m, 7H), 7.62 (dt, *J* = 1.2 and 8.4 Hz, 1H), 7.76 (t, *J* = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 27.3, 42.2, 60.1, 70.1, 115.3, 124.9, 126.8, 127.0, 127.5, 128.0, 128.6, 130.1, 130.6, 132.8, 133.1, 135.0, 136.8, 153.9, 158.6, 164.2. Anal. calcd. for $C_{24}H_{20}BrClN_2O_2$, %: C, 59.58; H, 4.17; N, 5.79. Found: C, 59.69; H, 4.35; N, 5.70.

Synthesis of 7-{2-{5-[4-(benzyloxy)phenyl]-3-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl}-2-oxoethoxy}-4-methyl-2*H*-chromen-2-one 6

A mixture of pyrazoline **5** (362.5 mg, 1 mmol), 4-methylumbelliferone (176 mg, 1 mmol) and KOH (66 mg, 85% purity, 1 mmol) in 96% ethanol (10 mL) was heated at reflux temperature for 3 h. The emulsion that resulted upon addition of water (40 mL) to the cold reaction mixture was extracted in chloroform (20 mL), the organic phase was washed with water (2 × 10 mL) and dried over anh. Na_2SO_4. Removal of chloroform under reduced pressure yielded a residue whose recrystallisation from ethyl acetate gave colourless crystals (290 mg, 50%), mp 193–194°C; ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 2.37 (d, J^4 = 1.2 Hz, 3H), 3.22 (dd, J_{AX} = 4.8 Hz and J_{AM} = 18.0 Hz, 1H), 3.77 (dd, J_{MX} = 11.6 Hz and J_{AM} = 18.0 Hz, 1H), 5.02 (s, 2H), 5.18 (d, *J* = 15.6 Hz, 1H), 5.23 (d, *J* = 15.6 Hz, 1H), 5.58 (dd, J_{AX} = 4.8 Hz and J_{MX} = 11.6 Hz, 1H), 6.12 (d, J^4 = 1.2 Hz, 1H), 6.81 (d, *J* = 2.4 Hz, 1H), 6.88–6.98 (m, 3H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.28–7.51 (m, 8H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.77 (s, 1H). ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 18.7, 41.7, 60.2, 66.2, 70.0, 101.5, 112.1, 113.1, 114.0, 115.3, 124.9, 125.6, 126.7, 127.1, 127.4, 128.0, 128.6, 130.2, 130.8, 132.5, 133.0, 135.0, 136.8, 152.5, 154.5, 155.1, 158.6, 161.2 (2 carbon atoms), 164.6. Anal. calcd. for $C_{34}H_{27}ClN_2O_5$, %: C, 70.52; H, 4.70; N, 4.84. Found: C, 70.21; H, 4.59; N, 5.03.

Synthesis of 4-[4-(benzyloxy)phenyl]-6-(3-chlorophenyl)pyrimidin-2-amine 7

To chalcone analogue **3** (697 mg, 2 mmol) and guanidine hydrochloride (286.5 mg, 3 mmol) in 96% ethanol (15 mL) a solution of KOH (395 mg, 6 mmol, 85% purity) in water (3 mL) was added, and the mixture was heated at reflux temperature for 6 h. Ethanol was removed under reduced pressure, the residue was triturated with water (50 mL), and the resulting mixture of emulsion and sticky semi-solid was left at room temperature over the week-end. The clear supernatant was then removed, and the residue was dried under vacuum overnight. One recrystallisation from 96% ethanol, followed by a second recrystallisation from 2-propanol of the residue recovered from the first recrystallisation, yielded a pale yellow solid (95 mg, 12%), mp 163–164°C. ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 5.15 (s, 2H), 5.18 (s, 2H), 7.08 (d, *J* = 8.8 Hz,

2H), 7.32–7.49 (m, 8H), 7.92 (d, *J* = 7.2 Hz, 1H), 8.01–8.09 (m, 3H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 70.1, 103.4, 115.1, 125.1, 127.3, 127.5, 128.1, 128.6, 128.7, 129.9, 130.0, 130.3, 134.9, 136.5, 139.7, 161.0, 163.4, 164.4, 165.9. Anal. calcd. for $C_{23}H_{18}ClN_3O$, %: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.51; H, 4.63; N, 10.60.

Synthesis of {3-[4-(benzyloxy)phenyl]oxiran-2-yl}(3-chlorophenyl)methanone 8

Chalcone analogue **3** (697 mg, 2 mmol) in acetone (20 mL) was sequentially treated with 30% hydrogen peroxide (3 mL) and 2N aq. NaOH (1 mL), and then the mixture was stirred at room temperature overnight. Water (80 mL) was gradually added to the vigorously stirred mixture, the resulting suspension was refrigerated for 1 h, and then the solid was filtered, washed with water, and air-dried. Recrystallisation from acetone–methanol (1:1 v/v) afforded a colourless solid (305 mg, 42%), mp 135–136°C. ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 4.02 (d, *J* = 1.6 Hz, 1H), 4.23 (d, *J* = 1.6 Hz, 1H), 5.10 (s, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.24–7.48 (m, 8H), 7.59 (dd, *J* = 0.8 and 8.0 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.99 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 59.5, 61.1, 70.1, 115.2, 126.5, 127.2, 127.3, 127.4, 128.1, 128.4, 128.7, 130.2, 133.9, 135.3, 136.6, 136.9, 159.6, 192.3.

Synthesis of 2-amino-5-[4-(benzyloxy)benzyl]-5-(3-chlorophenyl)-1*H*-imidazol-4(5*H*)-one 9

A mixture of oxirane **8** (237 mg, 0.65 mmol) and guanidine hydrochloride (93 mg, 0.97 mmol) in 96% ethanol (7 mL) was treated with 650 mg of a solution obtained by dissolving KOH (1 g, 85% purity) in 4 mL water, and then the mixture was heated at reflux temperature for 1 h. The solid that separated after the refrigeration of the mixture overnight was filtered, washed sequentially with water (5 mL) and 2-propanol (2 × 5 mL), and air-dried. The solid was heated in 96% ethanol at reflux temperature for 5 min, then the suspension was allowed to reach room temperature before being refrigerated for 2 h. The material was filtered and air-dried to give imidazolone **9** as a colourless solid (165 mg, 63%), mp 306–308°C; ^{1}H NMR (DMSO-d_6, 400.1 MHz), δ (ppm): 3.01 (dd, *J* = 13.2 Hz, 1H), 3.22 (dd, *J* = 13.2 Hz, 1H), 5.04 (s, 2H), 6.70–7.70 (br s, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.29–7.50 (m, 7H), 7.52–7.61 (m, 2H), 8.31 (s, 1H); ^{13}C NMR (DMSO-d_6, 100.6 MHz), δ (ppm): 42.7, 69.1, 70.2, 113.9, 124.3, 125.4, 126.9, 127.7, 127.8, 127.9, 128.4, 130.0, 131.2, 132.8, 137.1, 143.7, 157.1, 170.5, 187.2. Anal. calcd. for $C_{23}H_{20}ClN_3O_2$, %: C, 68.06; H, 4.97; N, 10.35. Found: C, 68.28; H, 5.08; N, 10.18.

Synthesis of 2-[4-(benzyloxy)phenyl]-4-(3-chlorophenyl)-2,3-dihydrobenzo[*b*]-1,4-thiazepine 10

Chalcone analogue **3** (348.5 mg, 1 mmol) and 2-aminothiophenol (150 mg, 1.2 mmol) were heated at reflux temperature in methanol (10 mL) in the presence of conc. HCl (5 drops) for 7 h. The mixture was kept in a refrigerator for 3 days, the crystalline mass was filtered, washed with a mixture of 2-propanol–hexanes (4 × 5 mL, 1:9 v/v), and air-dried. Two recrystallisations from small volumes of ethyl acetate afforded a light yellow solid (165 mg, 36%), mp 155–156°C. ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 3.03 (t, *J* = 12.8 Hz, 1H), 3.22 (dd, *J* = 4.8 and 13.2 Hz, 1H), 4.98 (dd, *J* = 4.8 and 12.0 Hz, 1H), 5.06 (s, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.15 (t, *J* = 7.3 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.32–7.44 (m, 6H), 7.47 (t, *J* = 6.8 Hz, 2H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 8.05 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 37.8, 60.1, 70.1, 115.0, 123.0, 125.4, 125.5, 125.6, 127.2, 127.4, 127.6, 128.0, 128.6, 129.8, 129.9, 130.9, 135.0, 135.1, 136.4, 136.8, 139.6, 152.0, 158.4, 167.6. Anal. calcd. for $C_{28}H_{22}ClNOS$, %: C, 73.75; H, 4.86; N, 3.07. Found: C, 73.56; H, 4.66; N, 2.95.

Reaction of chalcone analogue 3 with malononitrile in methanol in the presence of sodium methoxide

Chalcone analogue **3** (697 mg, 2 mmol) and malononitrile (132 mg, 2 mmol) were added to the solution obtained from sodium (46 mg, 2 gram-atom) in methanol (15 mL), and then the mixture was heated at reflux temperature for 6 h. The mixture was refrigerated overnight, and then the solid material was filtered, washed with cold 96% ethanol (2 × 5 mL), and air-dried. Column chromatography (ethyl acetate–hexanes 1:24 v/v) afforded first 4-[4-(benzyloxy)phenyl]-6-(3-chlorophenyl)-2-methoxypyridine **12** as a colourless solid (97 mg, 12%), mp 100–101°C, R_f = 0.5 (ethyl acetate–hexanes 1:24 v/v). ^{1}H NMR ($CDCl_3$, 400.1 MHz),

δ (ppm): 4.08 (s, 3H), 5.14 (s, 2H), 6.89 (d, *J* = 0.8 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.32–7.49 (m, 7H), 7.51 (d, *J* = 0.8 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.96 (dt, *J* = 2.0 Hz, 3.6 and 6.8 Hz, 1H), 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 53.5, 70.1, 107.0, 111.6, 115.3, 124.8, 127.0, 127.5, 128.1, 128.2, 128.7, 128.8, 129.8, 131.0, 134.7, 136.6, 141.1, 151.6, 153.5, 159.6, 164.6. Anal. calcd. for $C_{25}H_{20}ClNO_2$, %: C, 74.71; H, 5.02; N, 3.49. Found: C, 74.96; H, 4.78; N, 3.36.

Further elution allowed the isolation of 4-[4-(benzyloxy)phenyl]-6-(3-chlorophenyl)-2-methoxynicotinonitrile **11** as a colourless solid (188 mg, 22%), mp 169–170°C, R_f = 0.21 (ethyl acetate–hexanes 1:24 v/v). ^{1}H NMR ($CDCl_3$, 400.1 MHz), δ (ppm): 4.20 (s, 3H), 5.15 (s, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 7.32–7.50 (m, 8H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.96 (dt, *J* = 1.8 and 6.8 Hz, 1H), 8.08 (d, *J* = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100.6 MHz), δ (ppm): 54.7, 70.2, 93.3, 113.4, 115.3, 115.7, 125.3, 127.4, 127.5, 128.2, 128.5, 128.7, 129.9, 130.1, 130.3, 135.0, 136.4, 139.2, 156.2, 156.4, 160.4, 165.2. Anal. calcd. for $C_{26}H_{19}ClN_2O_2$, %: C, 73.15; H, 4.49; N, 6.56. Found: C, 73.38; H, 4.66; N, 6.39.

Synthesis of pyridine 11 through a multi-component approach

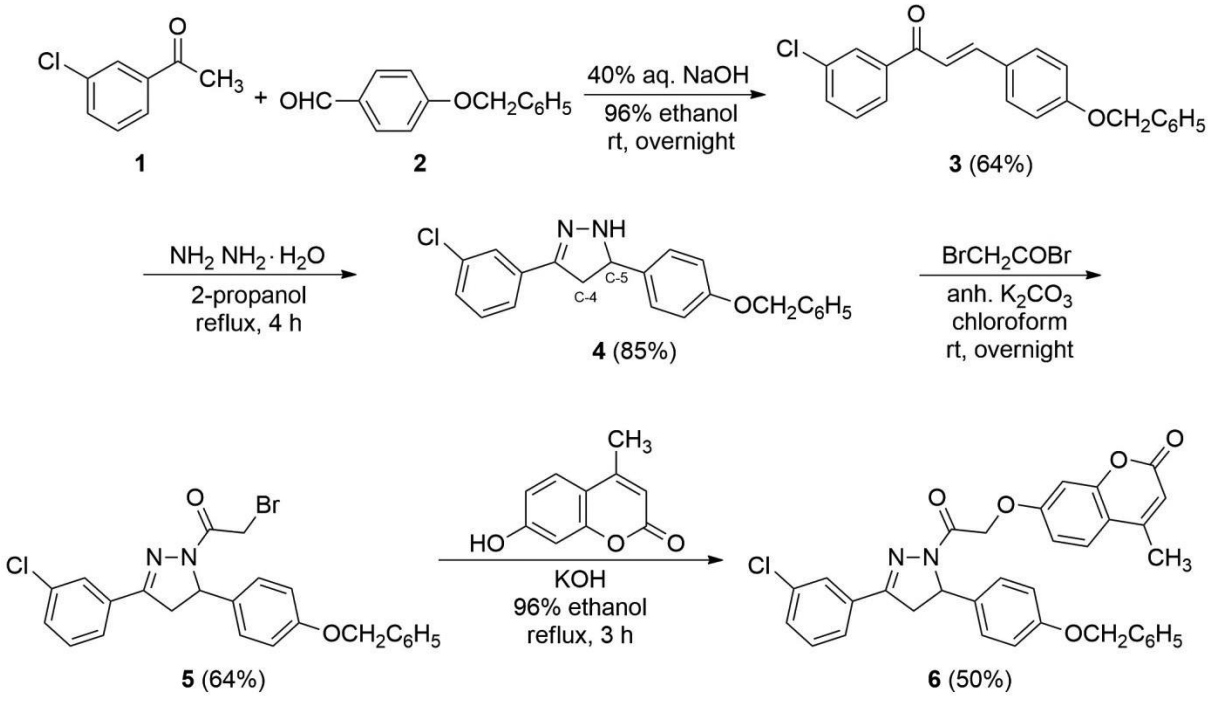
Sodium (23 mg, 1 gram-atom) was allowed to react with methanol (10 mL) at room temperature, then 4-(benzyloxy)benzaldehyde **1** (212 mg, 1 mmol), 3-chloroacetophenone **2** (154.5 mg, 1 mmol) and malononitrile (66 mg, 1 mmol) were added, and the mixture was heated at reflux temperature for 6 h. The material that separated upon cooling in a freezer overnight was filtered, washed with a mixture of hexanes–2-propanol (2 × 5 mL, 4:1 v/v), and air-dried to give a colourless solid (110 mg). Recrystallisation from 96% ethanol afforded colourless crystals (80 mg, 19%) with the same physical and spectroscopic characteristics as 4-[4-(benzyloxy)phenyl]-6-(3-chlorophenyl)-2-methoxynicotinonitrile **11** isolated in the previous procedure.

Examination of antimicrobial activity

The antimicrobial activity screening of the compounds in this study was performed using disk diffusion assay [15, 16] against three different reference strains, namely *S. aureus* ATCC25923, *E. coli* ATCC25922 and *C. albicans* ATCC10231. All microorganisms were stored at –80°C in 20% glycerol. The bacterial strains were refreshed on nutrient agar at 37°C, and the yeast strain was refreshed on Sabouraud dextrose agar also at 37°C. Microbial suspensions were prepared with these cultures in sterile solutions to obtain a turbidity that was optically comparable to that of 0.5 McFarland standards. Volumes of 0.1 mL from each inoculum were spread either onto nutrient agar or onto Sabouraud dextrose agar plates. After the medium surface had dried, sterilised paper discs (6 mm) were placed on the plate, and then aliquots (15 μL) of the tested compounds (concentration 100 mg/mL in DMSO) and DMSO as negative control were placed onto the paper discs. To evaluate the antimicrobial properties, the growth inhibition was measured under standard conditions after 24 h of incubation at 37°C. All tests were carried out in triplicate to verify the results. After incubation, the samples were analysed with SCAN1200®, version 8.6.10.0 (Interscience).

RESULTS AND DISCUSSION

The chalcone analogue **3** required as key intermediate for the generation of the heterocyclic compounds in the collection developed in this study was obtained through the Claisen–Schmidt condensation between 3-chloroacetophenone **1** and 4-(benzyloxy)benzaldehyde **2** (Scheme 1). Given the fair solubility of the starting materials in ethanol, the synthetic variant that takes place in this solvent and is catalysed by aqueous NaOH was preferred to the approach that uses piperidine as the basic catalyst and is conducted in refluxing methanol [17]. The structure of chalcone analogue **3** has been established using NMR analysis. The main characteristic of the proton NMR spectrum of compound **3** is the presence of two doublets with coupling constants of 15.2 Hz, assigned to the *trans* protons at the newly formed double bond in the α, β-enone fragment. While one of these doublets can be easily identified at 7.80 ppm, the chemical shift of the other has been determined through correlation spectroscopy to be centred at 7.50 ppm. The sharp singlet associated with the protons in the methylene group of the benzyl moiety appears at 5.13 ppm. 2D NMR experiments also allowed the identification of the peaks corresponding to the carbon atoms involved in the double bond of the α, β-enone fragment at 119.2 ppm and 145.5 ppm.



Scheme 1. Reaction sequence leading to pyrazoline–coumarin hybrid **6**

The structure of chalcone analogue **3** was further examined using single crystal X-ray diffraction, and the main crystallographic data from this investigation together with the refinement details are summarised in the Table. Interatomic distances and angles of chalcone analogue **3** are presented in Table S1 in Electronic Supplementary Information. X-ray diffraction analysis showed that compound **3** crystallises in the triclinic crystal system with the *P-1* space group (Fig. 1). The X-ray diffraction data indicated that the molecules of chalcone analogue **3** have a non-planar configuration, with dihedral angles between the either planes I (labelled as C1–C6) or planes II (labelled as C17–C22) of the peripheral phenyl rings and the plane of the central phenyl ring (labelled as C8-C13) of 100.18(7) and 21.57(7)°, respectively. Also, the geometric parameters of unit cell are quite similar to those found for reported chalcone analogues derived from 4-(benzyloxy)benzaldehyde [18]. In its crystal, compound **3** is stabilised by π–π stacking interactions with centroid-to-centroid distances of 3.8604(15) Å that involve the central phenyl rings in adjacent molecules (Fig. 2). The molecular packing of compound **3** in the crystal along *a* axis is shown in Fig. 3. The current data adds to the available information on the crystal structures of other chalcone analogues derived from 4-(benzyloxy)benzaldehyde that were synthesised and inves-

Table. **Crystal data and details of data collection**

	Compound **3**
empirical formula	$C_{22}H_{17}ClO_2$
Fw	348.80
space group	*P-1*
a [Å]	8.2641(7)
b [Å]	9.5978(9)
c [Å]	12.4439(9)
α [°]	108.233(7)
β [°]	94.307(6)
γ [°]	109.333(8)
V [Å^3]	866.84(13)
Z	2
ρ_{calcd} [g·cm^{-3}]	1.336
crystal size [mm]	0.20 × 0.15 × 0.10
T [K]	293(2)
μ [mm^{-1}]	0.232
2Θ range	3.516 to 50.052
reflections collected	7540
independent reflections	3073 [R_{int} = 0.0301]
data/restraints/parameters	3073/0/227
R_1	0.0437
wR_2	0.1125
GOF	1.058
Largest diff. peak/hole [e·Å^{-3}]	0.17/–0.21
CCDC no.	2357664

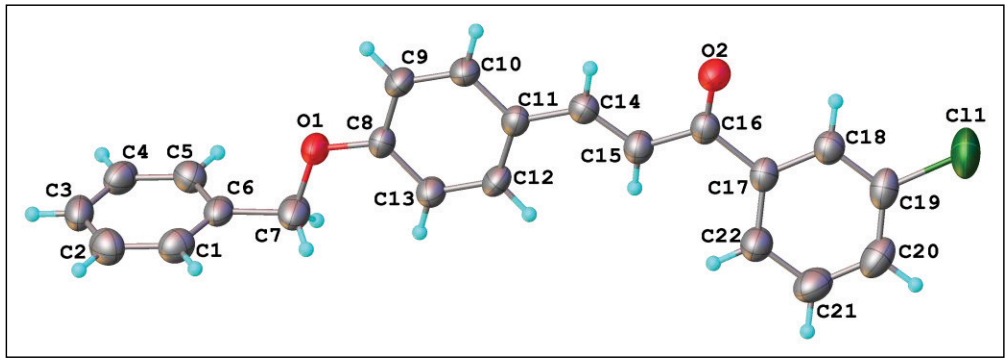


Fig. 1. X-ray molecular structure of compound **3** with atom labelling and thermal ellipsoids at 50% level

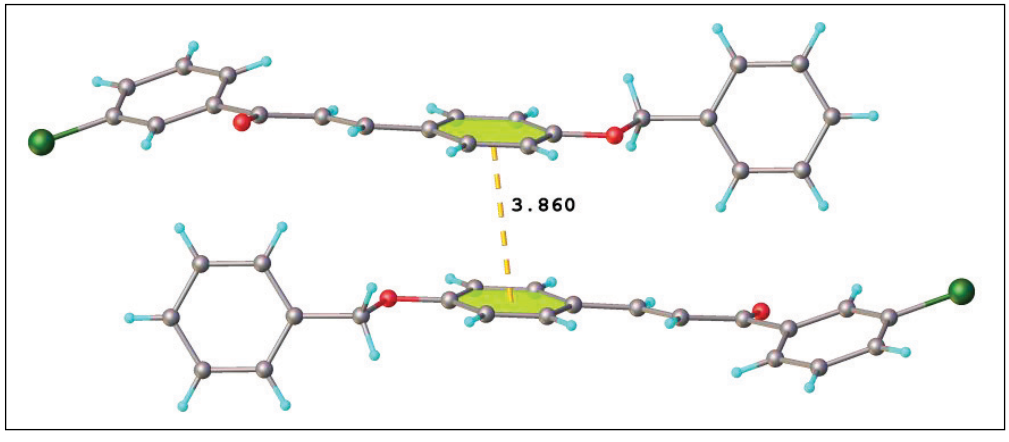


Fig. 2. π–π stacking interactions in the crystal structure of chalcone analogue **3**

tigated from a structural point of view in connection to their non-linear optical properties [19–21].

The reaction of the two electrophilic centres in the α, β-enone motif in chalcone analogues with bifunctional nucleophiles allows the generation of various types of heterocyclic compounds through a cyclocondensation process [22]. Hydrazine and monosubstituted hydrazines likely

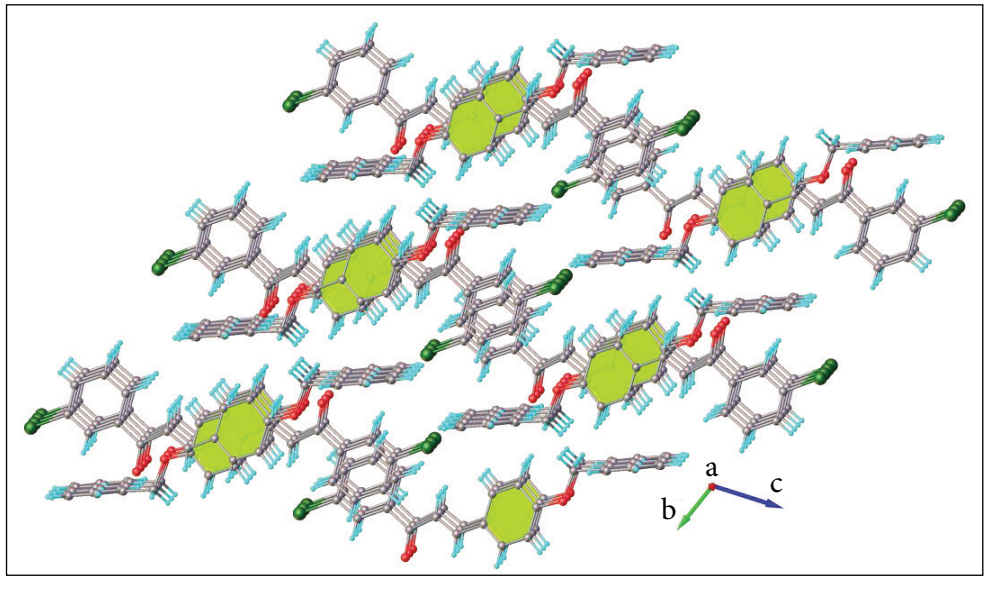
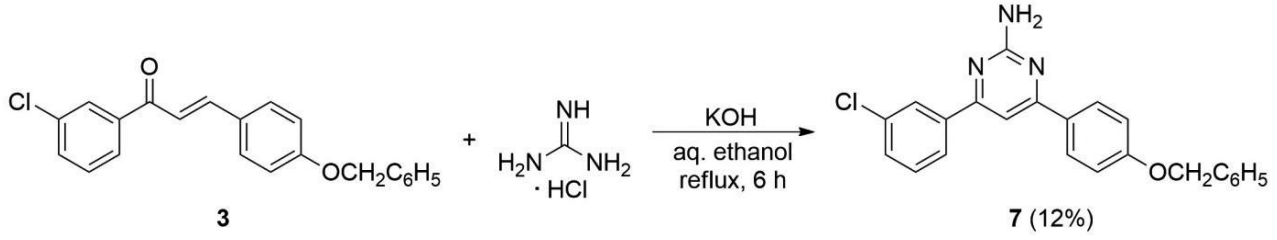


Fig. 3. The crystal packing diagram for compound **3** viewed along the *a* axis

represent the most common type of bifunctional nucleophile employed in the aforementioned cyclocondensations, and the use of these hydrazines afford either N-1 unsubstitued 2-pyrazolines or 2-pyrazolines with various substituents at N-1 [23]. While the antimicrobial activity of pyrazoline hybrids having a coumarin moiety at C-3 of the pyrazoline ring has been investigated [24, 25], no study on the antimicrobial activity of pyrazolines featuring a coumarin moiety at N-1 has been yet published, to the best of our knowledge. With the aim of preparing such a coumarin–pyrazoline hybrid **6**, a multi-step synthetic plan was devised for the coupling of the pyrazoline scaffold with the coumarin moiety through an ethanone fragment (Scheme 1). In the first step of the reaction sequence, chalcone analogue **3** was reacted with a twofold molar excess of hydrazine hydrate in 2-propanol at reflux temperature, according to a synthetic procedure previously reported for this type of compounds [26], an approach which afforded pyrazoline **4** with good yields (Scheme 1). Because of its potential lack of stability over time [27], intermediate **4** was structurally characterised by NMR in a timely manner and without prior purification through recrystallisation. The purity of the sample was nevertheless high, and allowed the identification of the NMR features that are typical for pyrazolines. Three groups of signals for the mutually coupled, magnetically non-equivalent protons C5-H_X, C4-H_A and C4-H_M that form an AMX three-spin system [28] and a doublet for the proton of the nitrogen atom (NH) have been identified in the proton spectrum of compound **4**. The peak for the carbon atom involved in the C=N double bond appears at about 147 ppm, the carbon atom in pyrazoline's methylene group gives a peak at approximately 40 ppm, while the signal for the carbon atom in pyrazoline's methine group appears at about 63 ppm. The second step in the reaction sequence towards coumarin–pyrazoline hybrid **6** dealt with the installment of the linker between the two scaffolds in the structure of this hybrid through the reaction of N-1 unsubstituted pyrazoline **4** with excess bromoacetyl bromide in chloroform, in the presence of K_2CO_3, at room temperature overnight. Processing of the reaction mixture led to the isolation in moderate yield (Scheme 1) of the desired compound, whose NMR characterisation confirmed its structure. In addition to the features related to the pyrazoline ring that have been previously outlined for intermediate **4** (the AMX three-spin system associated with protons C5-H_X, C4-H_A and C4-H_M), the ^{1}H NMR spectrum of compound **5** also presented two doublets at 4.30 and 4.34 ppm with a geminal coupling constant of 10.4 Hz typical for an AB spin system, which was attributed to the two protons of the bromoacetyl group. The peaks for the carbon atom of the methylene group and for the carbon atom of the carbonyl function in the bromoacetyl moiety have also been identified in the ^{13}C NMR spectrum of pyrazoline **5** at about 27 ppm for and at 164 ppm, respectively. Finally, the grafting of the coumarin fragment onto the pyrazoline scaffold was accomplished by displacement of the easily leaving bromine atom by 4-methylumbelliferone in the presence of KOH in refluxing ethanol (Scheme 1) to afford the target compound **6** with moderate yield. The presence of the coumarin fragment in the structure of hybrid **6** was evidenced in its proton NMR spectrum by the doublet at 2.37 ppm that was assigned to the protons in the methyl group and by the doublet at 6.12 ppm for the proton at C-3 in the coumarin ring system. Interestingly, the multiplicity of the signals in the proton NMR of compound **6** for the aforementioned, spatially proximal protons suggests that they are involved in long-range coupling.

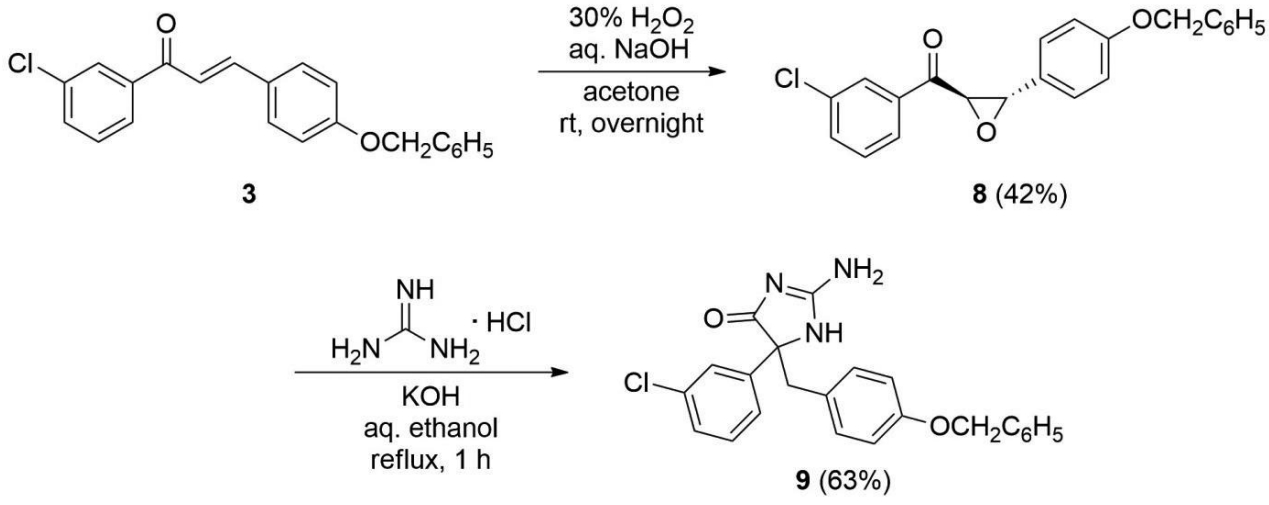
Another cyclocondensation reaction of chalcone analogue **3** that has been explored in this study involved guanidine as an example of bifunctional nucleophile. The use of guanidinium salts under basic reaction conditions and in the presence of air affords 2-aminopyrimidines [29, 30] through the oxidation of the intermediate dihydropyrimidine cycloadducts, which have been occasionally isolated as the main reaction product [31, 32]. When heated in ethanol in the presence of KOH at reflux temperature for 6 h, chalcone analogue **3** and guanidine hydrochloride afforded only a poor yield of the expected 2-aminopyrimidine **7** (Scheme 2), which could be isolated in a pure form from a complex mixture of reaction products after two recrystallisations from low boiling point primary alcohols. In the proton spectrum of pyrimidine derivative **7**, the sharp singlet at 5.15 ppm corresponds to the protons of the methylene group from the benzyl moiety,



Scheme 2. Conversion of chalcone analogue **3** into pyrimidine **7**

while the broad singlet at 5.18 ppm corresponds to the protons of the amino group. Although these two peaks are almost superimposed and have identical integral values, their assignment was unambiguously accomplished using H, C heteronuclear correlation spectroscopy. Thus, in the HSQC spectrum, the proton resonance from 5.18 ppm does not present any correlations, while the proton resonance from 5.15 ppm gives a correlation peak with the peak at 70.1 ppm that was associated with the methylene carbon of the benzyl moiety. The proton from C-5 of the pyrimidine ring was found overlapped by other aromatic protons peaks at 7.41 ppm. Its assignment was resolved based on long-range correlations in the HMBC spectrum of compound **7** with the peaks at 164.4 and 168.9 ppm, corresponding to C-4 and C-6 of the pyrimidine ring, respectively. Long-range correlations for the protons in the amino group could not be observed owing to the broad nature of the signal in the proton spectrum. Because they have no other protons in their vicinity except for those in the amino group, C-2 and C-5 of the pyrimidine ring are the only carbon atoms that give no correlation peaks in the HMBC spectrum. Nevertheless, C-5 can be associated with the peak at 103.4 ppm based on its direct correlation in the HSQC spectrum of pyrimidine **7** with the signal at 7.41 ppm for the proton at C-4, while the remaining peak at 134.9 ppm was consequently attributed to C-2.

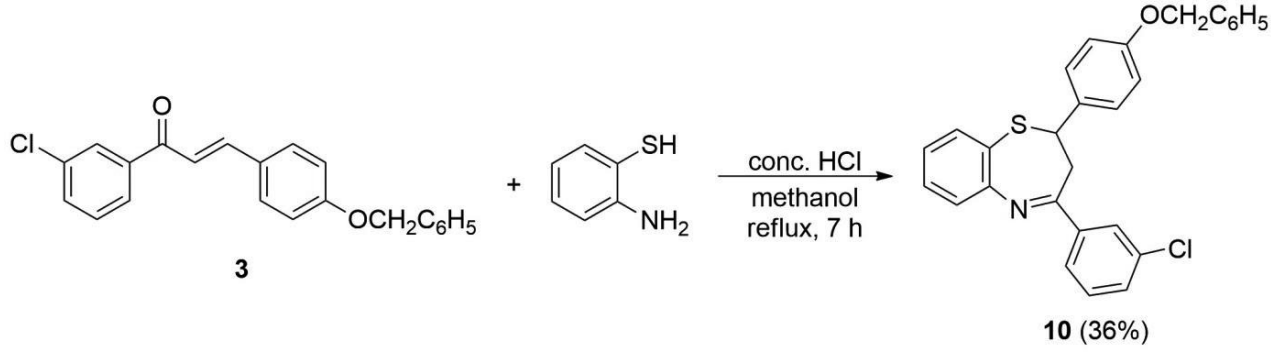
In an attempt to increase the yield of pyrimidine **7**, the cyclocondensation of chalcone analogue **3** and guanidine hydrochloride in the presence of hydrogen peroxide as an oxidising agent [33] for the conversion of intermediate dihydropyrimidine into its fully aromatic counterpart was also examined. Unfortunately, the careful duplication of the experimental approach detailed in the aforementioned work led to amounts that were similar to those obtained in the absence of the oxidising agent. However, a sample of compound **7** obtained under these reaction conditions retained an impurity that did not dissolve in $CDCl_3$ for NMR analysis, while the pure pyrimidine **7** prepared in the absence of the oxidising agent did. The sample did dissolve completely in DMSO-d_6, and its proton NMR spectrum presented one set of peaks for a major component that were attributed to pyrimidine **7**, and a second set of signals for an impurity (ratio pyrimidine **7**:impurity of approximately 2.5:1, based on the signal of the protons in the methylene group of the benzyl fragment). Because the study reporting the synthesis of 2-aminopyrimidines from chalcone analogues and guanidinium chloride in the presence of hydrogen peroxide also showed that a change in the order of addition of the reagents could lead to 5,5-disubstituted-2-amino-4,5-dihydroimidazol-4-ones instead, it was hypothesised that the impurity in our sample is 2-amino-5-[4-(benzyloxy)benzyl]-5-(3-chlorophenyl)-1*H*-imidazol-4(5*H*)-one **9**. In order to prove that our hypothesis is correct, and also faced with the opportunity of adding another type of heterocycle (and one that has been only scarcely reported in the literature, for that matter) to the library generated from chalcone analogue **3**, a stepwise synthetic approach (Scheme 3) was implemented for the controlled, exclusive preparation of imidazolone **9**. The first step in this approach was the preparation of oxirane **8** through the Weitz–Scheffer epoxidation of chalcone analogue **3** in acetone, a process that proved to be so sluggish compared to previous similar epoxidations performed by our group [34] that the reaction time had to be extended from 1 to 20 h to ensure a satisfactory conversion of the substrate. The characteristic signals in the proton NMR spectrum of chalcone epoxide **8** are the two doublets 4.02 and 4.23 ppm that have a value of the coupling constants of 1.6 Hz, which is indicative of the *trans* configuration of the substituents in



Scheme 3. Preparation of imidazolone **9** through oxirane intermediate **8**

the oxirane ring [35]. The two signals corresponding to the carbon atoms in the oxirane ring were identified at 59.5 and 61.1 ppm in the carbon spectrum of chalcone epoxide **8**. Ring-opening of this oxirane with guanidine hydrochloride afforded in a smooth manner (albeit in moderate yield) a sole compound, whose NMR analysis confirmed that the substance was the desired imidazolone **9**. Thus, the AB doublets of the protons in the methylene group adjacent to the chiral carbon atom in the imidazolone ring appear at 3.01 and 3.22 ppm, the protons in the exocyclic amino group give a very broad singlet that overlaps the distinct peaks of the aromatic protons, while the proton of the endocyclic nitrogen gives a sharp singlet at 8.31 ppm. An additional NMR experiment that was performed at 55°C allowed the discrimination of the signal associated with the protons of the amino group from the signals of the aromatic protons as a relatively broad singlet centred at 7.01 ppm. The quaternary sp^3 carbon in the 3,5-dihydroimidazol-4-one ring in compound **9** was identified at 70.2 ppm, while the carbon atom in the carbonyl group of imidazolone was assigned the signal at 187.2 ppm.

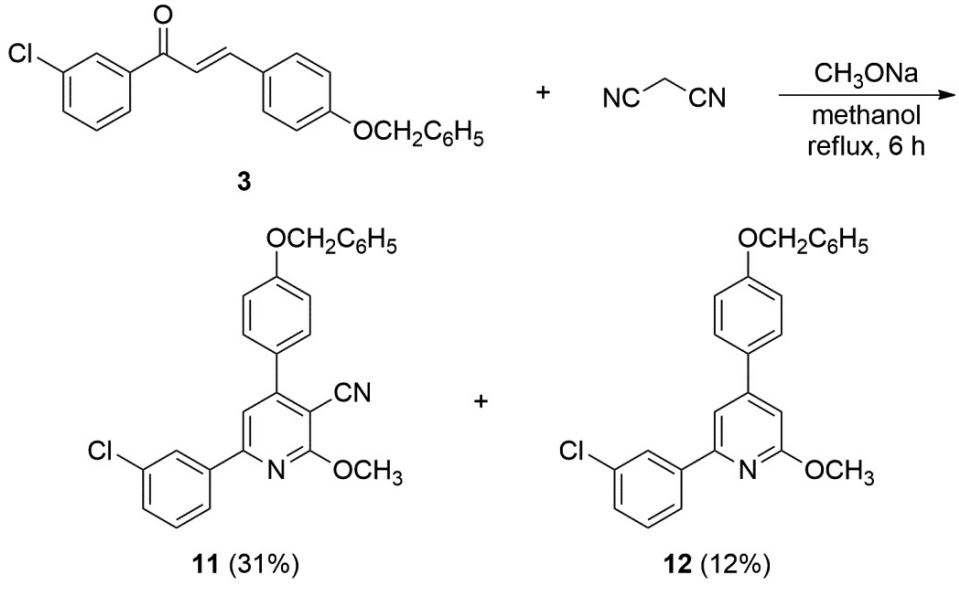
Ring-closure reaction of chalcone analogues with *o*-aminothiophenols affords dihydrobenzothiazepines [36, 37], many of them being synthesised with the aim of investigating their antimicrobial activity [38–40]. Benzothiazepine derivative **10** was isolated in 36% yield from the cyclocondensation of chalcone analogue **3** with 2-aminobenzenethiol under previously reported [41] reaction conditions (refluxing methanol in the presence of conc. HCl as catalyst, Scheme 4). Its formation was supported by NMR spectroscopy through the existence of three sets of signals at 3.03, 3.22 and 4.98 ppm in the proton NMR spectrum of compound **10** that correspond to the aliphatic protons in the dihydrobenzothiazepine moiety. The presence of two peaks at 37.8 and 60.1 ppm in the aliphatic region of the carbon spectrum of the same compound confirms the 1,4-addition of the mercapto group to



Scheme 4. Synthesis of benzothiazepine derivative **10**

the α, β-enone motif [3]. The ring closure to benzothiazepine derivative **10** is supported by the absence of a peak close to 190 ppm (similar to that found in thia-Michael adducts [3] of 4-chlorothiophenol to chalcone analogues), and by the presence of a peak at 167.6 ppm that was assigned using 2D NMR spectroscopy to the carbon involved in the double carbon–nitrogen bond in the dihydrobenzothiazepine moiety.

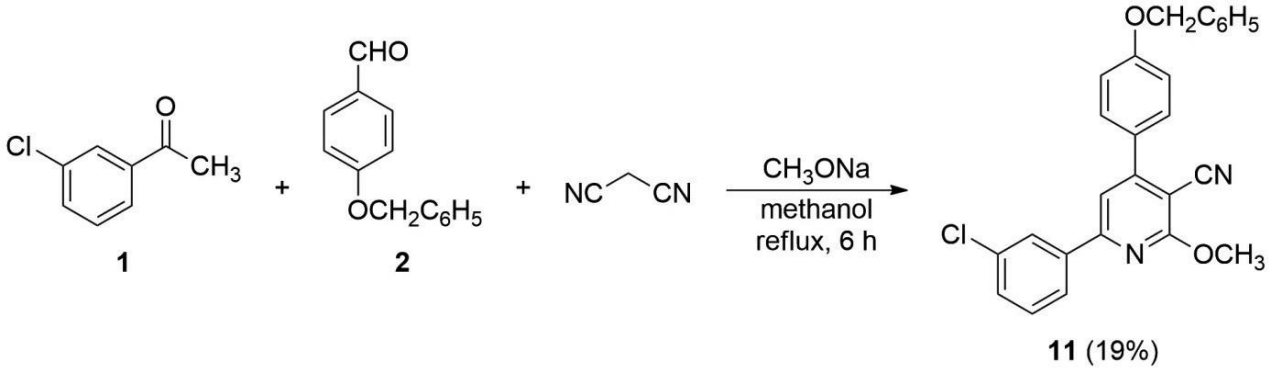
Reaction of chalcone analogues with malononitrile in alcohols in the presence of the corresponding sodium alcoxide provides a one-step entry to nicotinonitriles substituted at position 2 with an alcoxy moiety deriving from the alcohol employed as a solvent [42, 43]. When a solution of chalcone analogue **3** in methanol was heated at reflux temperature with malonitrile and sodium methoxide for 6 h (Scheme 5), the solid material that was isolated consisted of a mixture of two compounds based on the presence in the proton spectrum of two distinct singlets in the range of 4.0–4.2 ppm (that were tentatively attributed relying on the assignments reported in previous studies [42, 43] to protons in methoxy groups). In addition, the occurrence of two almost superimposed singlets at approximately 5.14 ppm that were associated to the protons in the methylene group of the benzyloxy fragment suggested the presence of two components in the sample. The ratio between these two compounds in the mixture, as it was determined using the integration values for the well-segregated pair of signals for the protons in the methoxy groups, was 1:0.55. Separation of this mixture using column chromatography allowed the isolation of the pure components **11** and **12** (Scheme 5), whose structure was then established using NMR spectroscopy. The major component **11** was the expected 2-methoxynicotinonitrile, for which the singlet at 4.20 ppm in its proton NMR spectrum was indicative of the presence of the protons of the methoxy group, while the peak at 54.7 ppm in its ^{13}C NMR spectrum was proof for the presence of the carbon atom in the same group. Also, the peak at 93.4 ppm in its ^{13}C NMR spectrum constitutes evidence for the existence of the cyano group. The minor component **12** was ascribed to the structure of 2-methoxypyridine. The grounds for this particular structural designation for compound **12** relied on the identification of signals for one extra proton in the aromatic region of its NMR spectrum, and also by the absence of a peak associated with the carbon atom of the cyano group of its carbon NMR spectrum. Thus, an additional doublet with a coupling constant of 0.8 Hz and integrating for one proton was observed in the proton spectrum of pyridine **12** at 6.89 ppm. Homonuclear correlation spectroscopy revealed the coupling of this extra doublet with the doublet at 7.51 ppm, which also has a coupling constant of 0.8 Hz. Since the rest of the signals were almost identical with the ones that have been fully assigned in the spectrum of nicotinonitrile **11**, the aforementioned doublets



Scheme 5. Cyclocondensation of chalcone analogue **3** with malononitrile leading to pyridines **11** and **12**

were assumed to belong to the protons at C-3 and C-5 of the 2-methoxypiridine moiety. Heteronuclear direct correlation spectroscopy revealed that these protons were bound to those carbon atoms that give the peaks at 106.9 and 111.6 ppm, respectively. Using long-range heteronuclear correlation spectroscopy, the peaks from 7.51 and 111.6 ppm were associated with the H-5 and C-5 of the 2-methoxypiridine moiety, while the peaks at 6.89 and 106.9 ppm were allocated to the H-3 and C-3 of the 2-methoxypiridine moiety. Also, both H-3 and H-5 showed long-range correlations with each other and with the 4-benzyloxyphenyl moiety in the HMBC spectrum, while H-5 additionally gave long-range correlations with the chlorine-substituted phenyl ring. The preparation of analogues of 2-methoxypyridine **12**, either as sole reaction products or in mixtures with the structurally related nicotinonitriles, has been recently reported [44] to arise from the cyclocondensation of chalcone analogs with malononitrile conducted in methanol in the presence of sodium methoxide at room temperature. According to this study, 2-methoxypyridines analogous to **12** are by-products obtained through the extrusion of HCN from the intermediate dihydropyridines, while nicotinonitriles **11**, which have been overwhelmingly reported as the usual reaction products in such cyclocondensation reactions, derive from the dehydrogenation of the same intermediate dihydropyridine [44]. Because it was found that 2-methoxypyridine **12** is more soluble in 96% ethanol than nicotinonitrile **11**, the removal of the former from the mixture of components directly isolated from the work-up of the reaction mixture could also be achieved through two sequential recrystallisation processes, affording facile access to pure compound **11** with yields (31%) that are slightly superior to those recorded after the chromatographic separation (22%). Furthermore, a survey of the literature dealing with the synthesis of analogues of nicotinonitrile **11** revealed that these compounds may also be prepared in a one-pot, multi-component reaction using an aryl methyl ketone, an aromatic aldehyde, malononitrile and alcoxide as starting materials [45, 46]. Therefore, the outcome of a process in which 3-chloroacetophenone **1**, 4-(benzyloxy)benzaldehyde **2**, malononitrile and sodium methoxide were reacted in methanol (Scheme 6) was also examined. The solid that was isolated after work-up consisted only of the desired nicotinonitrile **11** in a pure form (as estimated by NMR) with yields that are comparable with the synthetic approach starting from chalcone analogue **3**. These results show that, at least in this case, the multi-component method provides a better way to obtain compound **11** in a single stage from compounds **1** and **2** compared to the variant employing chalcone analogue **3** as the intermediate that must be beforehand prepared from the same starting materials **1** and **2**.

A preliminary examination of the antimicrobial activity for the heterocyclic compounds in this study has been also performed. The antimicrobial susceptibility of the compounds was evaluated using the Kirby–Bauer agar disk diffusion test, which involves the addition of the compounds on the culture medium pre-inoculated with the microbial suspension, and measuring the clear zone caused by the growth inhibition around the disks after 24 h of incubation. The compounds selected for this evaluation were heterocycles **6**, **7** and **9**–**12**, while one Gram-positive bacterial strain (*S. aureus*), one Gram-negative bacterial strain (*E. coli*) and one yeast (*C. albicans*) were employed



Scheme 6. Multi-component process yielding nicotinonitrile **11** as the sole reaction product

as representative microorganisms for these specific types of microorganisms. None of the compounds presented antimicrobial activity against the tested reference strains at a concentration of 100 mg/mL.

CONCLUSIONS

A novel chalcone analogue was successfully synthesised with good yields and structurally characterised, then its reactivity in the preparation of structurally diverse heterocycles was examined. An example of a structural type of pyrazoline–coumarin hybrid that has not been yet reported in the literature was prepared from this α, β-enone in a total yield of 27% after three steps, out of which the first step, namely the cyclocondensation of the chalcone analogue with hydrazine, produced the N^1-unsubstituted pyrazoline with 85% yield. Only a low yield of 2-aminopyrimidine was obtained through the cyclocondensation of the chalcone analogue with guanidine under reaction conditions that have been previously reported to afford good to excellent yields of pyrimidine derivatives, and the use of an oxidising agent with a view to promote the conversion of the intermediate dihydropyrimidine into the fully aromatised pyrimidine did not result in improved yields of the desired product, but instead gave imidazolone as by-product of a side reaction. The same imidazolone could be exclusively prepared in good yields via a process that employed the epoxide of the chalcone analogue as an intermediate. The benzothiazepine derivative arising from the ring closure reaction of the chalcone analogue with 2-aminobenzenethiol was also obtained with moderate yields only. From the cyclocondensation of this chalcone analogue with malononitrile under previously employed reaction conditions, a mixture comprising nicotinonitrile as a normal reaction product and 2-methoxypyridine as a by-product, both in low yields, was obtained. A multi-component process could be alternatively used to produce nicotinonitrile as the sole reaction product, albeit in a moderate yield. All these results point out that this particular chalcone analogue has a lesser reactivity when compared to other chalcone analogues in the majority of the reactions leading to various heterocycles under standard reactions conditions that were examined in this study. The heterocycles derived from this chalcone analogue exhibited no noticeable antimicrobial activity even at high concentration. Because numerous compounds featuring the heterocyclic cores (i.e. pyrazoline, pyrimidine, benzothiazepine and pyridine) prepared in this study are known to have antibacterial activity, it may be hypothesised that the substitution of these heterocyclic scaffolds with the specific aromatic moieties derived from the chalcone analogue (namely, 3-chlorophenyl and 4-(benzyloxy)phenyl) does not impart to the resulting chemical entities any activity toward the microorganisms under investigation.

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IŠ 3-[(4-BENZILOKSI)FENIL]-1-(3-CHLORFENIL) PROP-2-EN-1-ONO GAUTŲ HETEROCIKLŲ SINTEZĖ IR ANTIMIKROBINIS TYRIMAS