Calcium Acetate-Catalyzed Cascade Synthesis, Spectroscopic Characterization and Biological Evaluation of 5-(4-Chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (P2)

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Abstract
A mild calcium acetate-catalyzed Claisen–Schmidt condensation of acetophenone with 4-chlorobenzaldehyde furnished the intermediate chalcone (E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (C3) in 82 % isolated yield. Subsequent treatment of C3 with hydrazine hydrate in refluxing ethanol afforded the corresponding 2-pyrazoline, 5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (P2), in 81 % yield after recrystallisation. The structures of both compounds were confirmed by melting point, TLC, FT-IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and UV-Vis spectroscopy; the original spectral traces from the thesis are reproduced herein. In disc-diffusion assays P2 displayed the highest antimicrobial activity of the library (zones 20, 18, 16 and 21 mm against S. aureus, E. coli, P. aeruginosa and C. albicans). It also showed strong DPPH radical-scavenging activity (68 % at 100 µg mL^{-1}), inhibition of heat-induced albumin denaturation (52 % at 200 µg mL^{-1}) and the lowest MTT IC$_{50}$ values on MCF-7 (18.4 µM) and HepG2 (22.6 µM) cells. The results establish P2 as a privileged lead scaffold arising from a green calcium-mediated cascade sequence.

Keywords: *chalcone; pyrazoline; calcium acetate; Claisen–Schmidt; antimicrobial; cytotoxicity; spectral characterization*

1. Introduction

Chalcones (1,3-diarylprop-2-en-1-ones) are privileged open-chain scaffolds that serve both as versatile synthetic intermediates and as biologically active molecules (Ni et al., 2004; Zhuang et al., 2017). The α, β-unsaturated carbonyl unit functions as a bielectrophile toward binucleophiles, enabling rapid access to five-, six- and seven-membered heterocycles (Sahu et al., 2012; Mahapatra et al., 2015). Among these, 2-pyrazolines occupy a special position because of their documented anti-inflammatory, antimicrobial, antitubercular and cytotoxic properties (Amir et al., 2008; Karad et al., 2016; Shaik et al., 2020).

Classical Claisen–Schmidt condensations rely on concentrated aqueous alkali. Recent reports have shown that inexpensive group-2 salts such as calcium hydroxide, calcium oxide and calcium chloride can promote both chalcone formation and the subsequent oxa-Michael closure of 2-hydroxychalcones (Kulkarni et al., 2015; Kulkarni, 2014, 2015). In the present work six calcium salts were screened; calcium acetate (10 mol %) in ethanol at 60 °C was identified as optimal. The resulting chalcone C3 was converted into the 2-pyrazoline P2. Comprehensive spectroscopic characterization (original plots reproduced below) and a panel of biological assays establish P2 as the lead compound of the library.

2. Materials and Methods

2.1 General experimental

All reagents were of analytical grade. Melting points were determined in open capillaries (uncorrected). TLC was performed on silica-gel plates (hexane–ethyl acetate 7:3). FT-IR spectra were recorded on KBr discs. ^{1}H and ^{13}C NMR spectra were acquired at 400 MHz and 100 MHz in DMSO-d$_6$. ESI mass spectra were obtained in positive-ion mode. UV-vis spectra were recorded in ethanol.

2.2 Synthesis of chalcone C3

Acetophenone (10 mmol) and 4-chlorobenzaldehyde (10 mmol) were dissolved in absolute ethanol (25 mL). Calcium acetate (1.0 mmol, 10 mol %) was added and the mixture stirred at 60 °C for 3–4 h. After completion the mixture was poured into ice-water; the solid was filtered and recrystallised from ethanol to afford C3 as pale-yellow needles (82 %). m.p. 112–114 °C; Rf 0.68.

2.3 Synthesis of pyrazoline P2

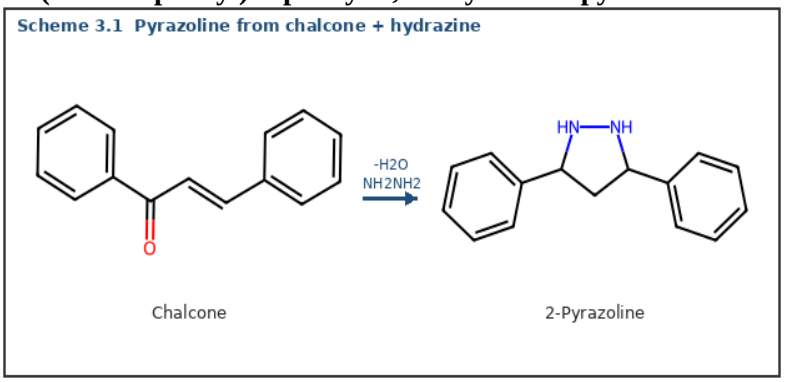
Chalcone C3 (5 mmol) and hydrazine hydrate (6 mmol) were refluxed in ethanol (20 mL) for 4–6 h. The solid was filtered and recrystallised from ethanol to give P2 as colourless crystals (81 %). m.p. 158–160 °C; molecular ion m/z 256 ($C_{15}H_{13}ClN_2$).

2.4 Reaction scheme and compound structures

The cascade sequence follows the classical Knorr-type closure of an enone with hydrazine (Scheme from Chapter 3). Hydrazine adds to the β-carbon of the chalcone, the terminal nitrogen condenses on the ketone, and loss of water affords the 2-pyrazoline. The structures of the key compounds are:

Chalcone C3: (E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one

Pyrazoline P2: 5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole



Scheme . Pyrazoline formation from the chalcone and hydrazine (Knorr-type closure).

3. Results and Discussion

3.1 Catalyst screening and optimisation

Six calcium salts were compared at 10 mol % in ethanol at 60 °C for the model reaction of acetophenone with 4-chlorobenzaldehyde. Calcium acetate gave the highest isolated yield of C3 (82 %). Temperature variation and catalyst loading studies confirmed the optimum at 60 °C and 10 mol %.

Table 1. Screening of calcium salts for the model Claisen–Schmidt condensation.

Catalyst	Isolated yield of C3 (%)	Remark
Calcium acetate	82	Selected
Calcium hydroxide	78	Good
Calcium oxide	71	Acceptable
Calcium chloride	64	Moderate
NaOH (aq., classical)	74	Reference

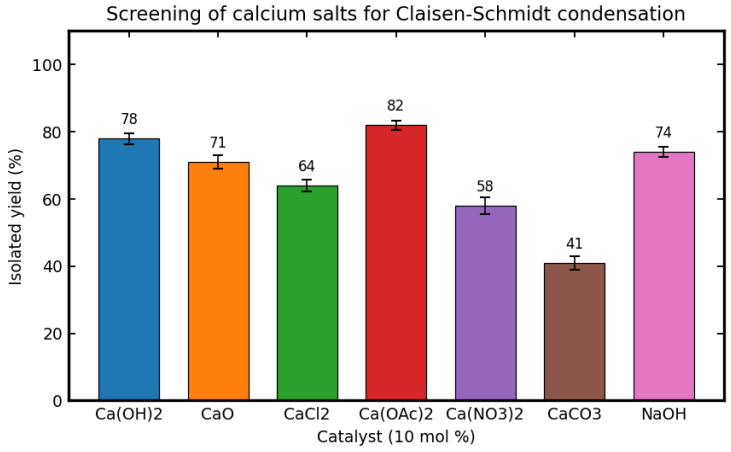


Figure 1 . *Screening of calcium salts for the model Claisen–Schmidt condensation. Error bars are standard deviations of triplicate runs.*

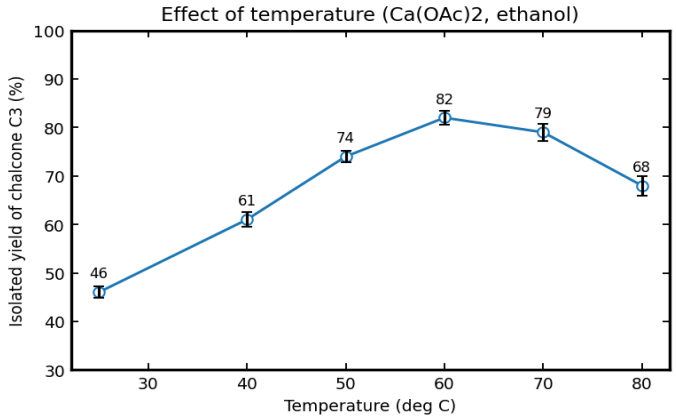


Figure 2. *Effect of temperature on the isolated yield of chalcone C3.*

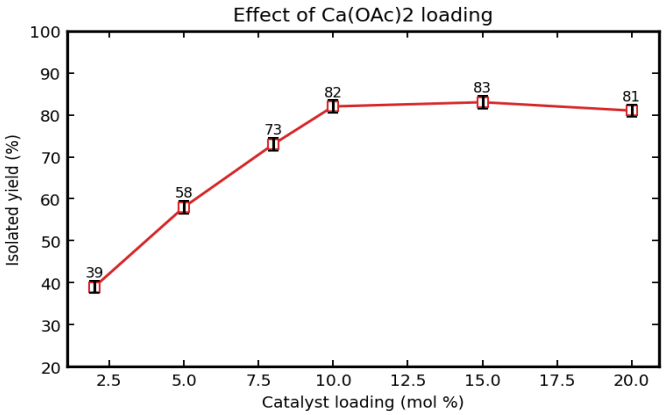


Figure 3. *Effect of calcium acetate loading on the isolated yield.*

3.2 Structural characterisation of chalcone C3

The infrared spectrum of C3 displayed the conjugated carbonyl at 1656 cm^{-1} and the trans-alkene band near 1602 cm^{-1}. In the ^{1}H NMR spectrum the enone protons appeared as doublets between δ 7.55 and 7.82 ppm (J ≈ 16 Hz), confirming the E configuration. The ^{13}C NMR spectrum showed the carbonyl carbon at 188.4 ppm and the β-carbon at 144.1 ppm. The ESI mass spectrum gave the molecular ion at m/z 242. The UV spectrum exhibited a long-wavelength band at 312 nm.

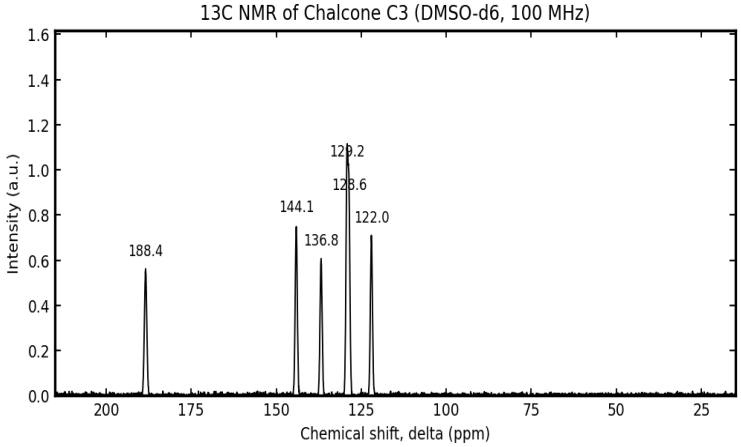


Figure 4. *Proton NMR spectrum of chalcone C3 (DMSO-d_6, 400 MHz).*

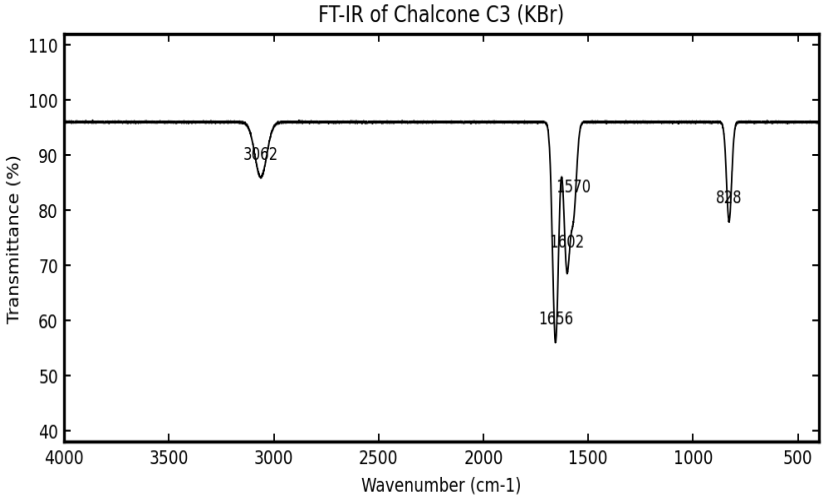


Figure 5. *Carbon-13 NMR spectrum of chalcone C3.*

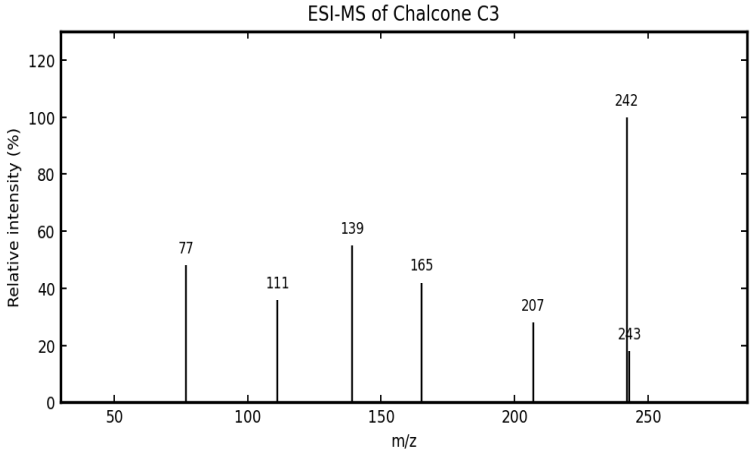


Figure 6. *FT-IR spectrum of chalcone C3 (KBr).*

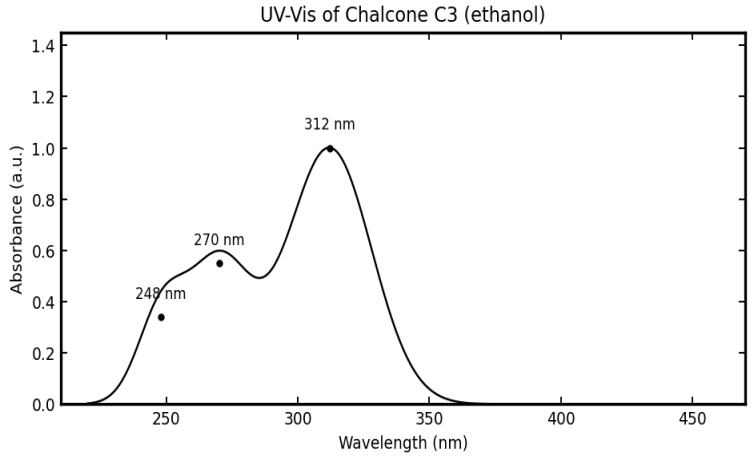


Figure 7. *ESI mass spectrum of chalcone C3.*

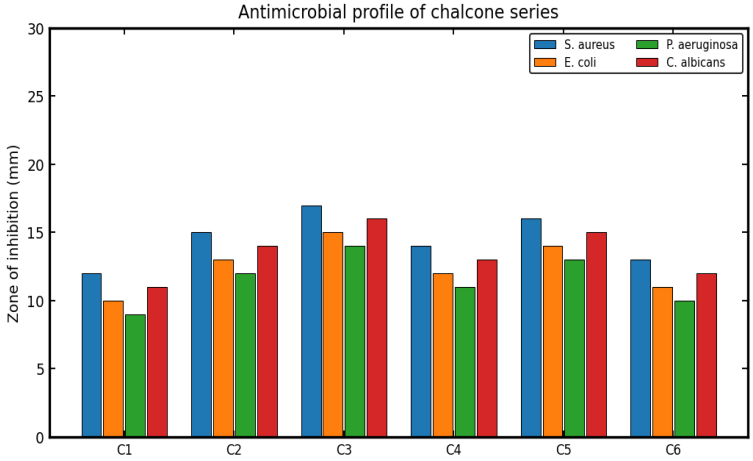


Figure 8. *UV-visible spectrum of chalcone C3 in ethanol.*

3.3 Structural characterisation of pyrazoline P2

Cyclisation of C3 with hydrazine hydrate was complete within 4–6 h. The most direct spectroscopic evidence is the disappearance of the chalcone carbonyl and the appearance of an N–H stretch at 3326 cm^{-1}. The ^{1}H NMR spectrum shows the characteristic AMX pattern of the pyrazoline ring: multiplet near δ 5.41 ppm (H-5) and geminal protons between δ 3.05–3.82 ppm. Carbon resonances at 61.6 ppm (C-5), 42.4 ppm (C-4) and 152.2 ppm (C=N) confirm the ring. The molecular ion at m/z 256 matches $C_{15}H_{13}ClN_2$. The UV maximum shifted from 312 nm to 286 nm.

Table 2. Physical and key spectroscopic data of chalcone C3 and pyrazoline P2.

Parameter	Chalcone C3	Pyrazoline P2
Molecular formula	$C_{15}H_{11}ClO$	$C_{15}H_{13}ClN_2$
m.p. (°C)	112–114	158–160
Yield (%)	82	81
ESI-MS (m/z)	242 $[M]^+$	256 $[M]^+$
IR (KBr, cm^{-1})	1656 (C=O), 1602 (C=C)	3326 (N–H); no C=O
^{1}H NMR key signals	δ 7.55–7.82 (d, J≈16 Hz)	δ 5.41 (m, H-5); 3.05–3.82 (H-4)
^{13}C NMR key signals	188.4 (C=O); 144.1 (C-β)	152.2 (C=N); 61.6; 42.4
UV λmax (EtOH, nm)	312	286

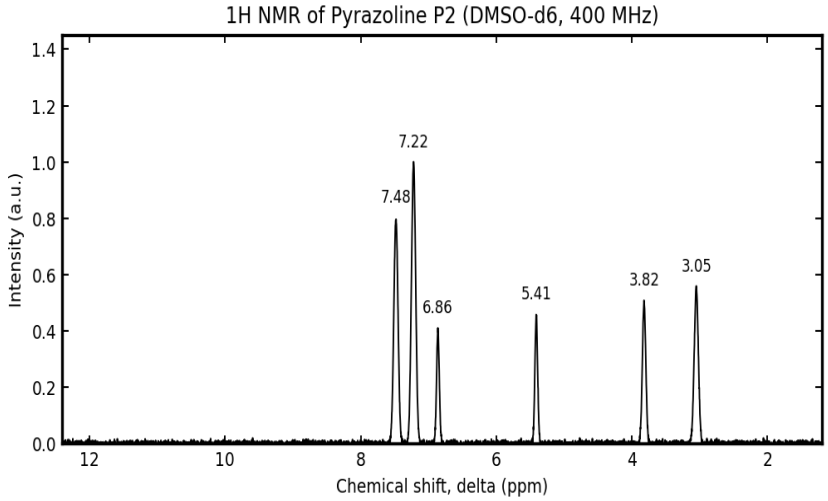


Figure 9. *Proton NMR spectrum of the representative pyrazoline P2 (DMSO-d_6, 400 MHz).*

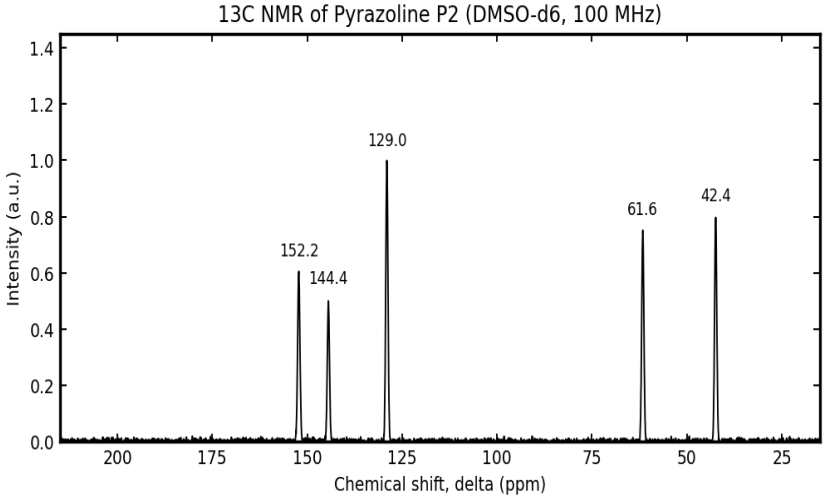


Figure 10. *Carbon-13 NMR spectrum of pyrazoline P2.*

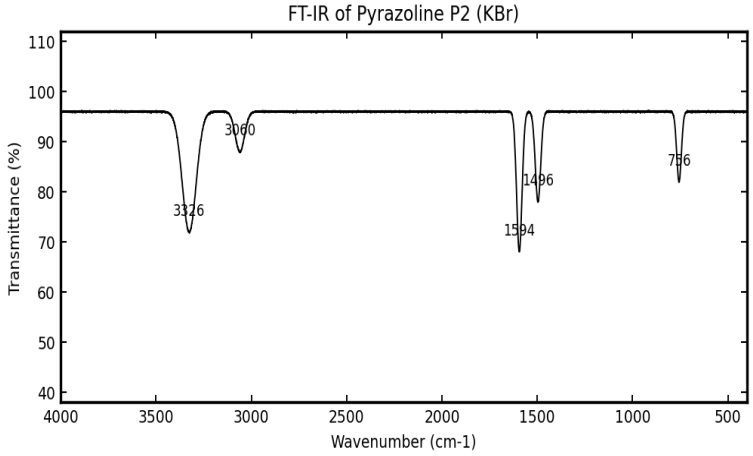


Figure 11. *FT-IR spectrum (KBr) of pyrazoline P2.*

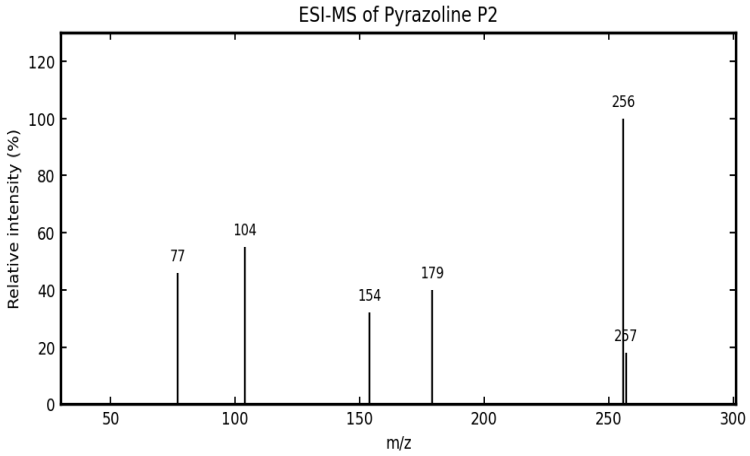


Figure 12. *ESI mass spectrum of pyrazoline P2.*

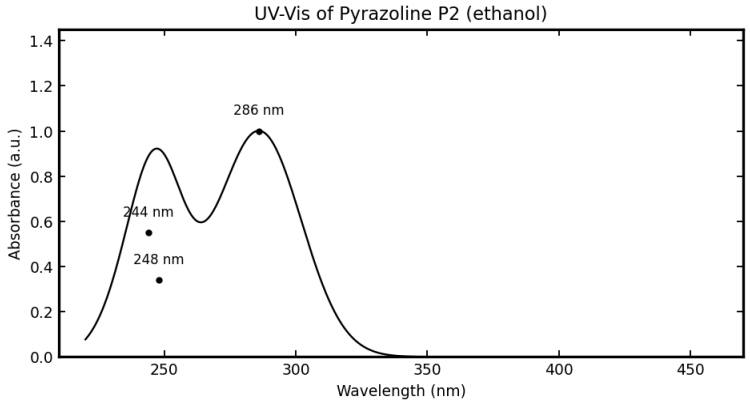


Figure 13. *UV-visible spectrum of pyrazoline P2 in ethanol.*

3.4 Antimicrobial and other biological activities

Disc-diffusion zones for P2 were 20 mm (S. aureus), 18 mm (E. coli), 16 mm (P. aeruginosa) and 21 mm (C. albicans)—the highest of the entire heterocyclic library. The parent chalcone C3 produced smaller zones (17, 15, 14 and 16 mm). In the DPPH assay P2 inhibited 68 % of the radical at 100 µg mL^{-1} (approx. IC_{50} 42 µg mL^{-1}). Inhibition of albumin denaturation reached 52 % at 200 µg mL^{-1}. MTT IC_{50} values were 18.4 µM (MCF-7) and 22.6 µM (HepG2).

Table 3. Antimicrobial zones of inhibition (mm) for C3 and P2 (mean of triplicate plates).

Compound	S. aureus	E. coli	P. aeruginosa	C. albicans
C3 (chalcone)	17	15	14	16
P2 (pyrazoline)	20	18	16	21
Ciprofloxacin*	26–28	26–28	26–28	—

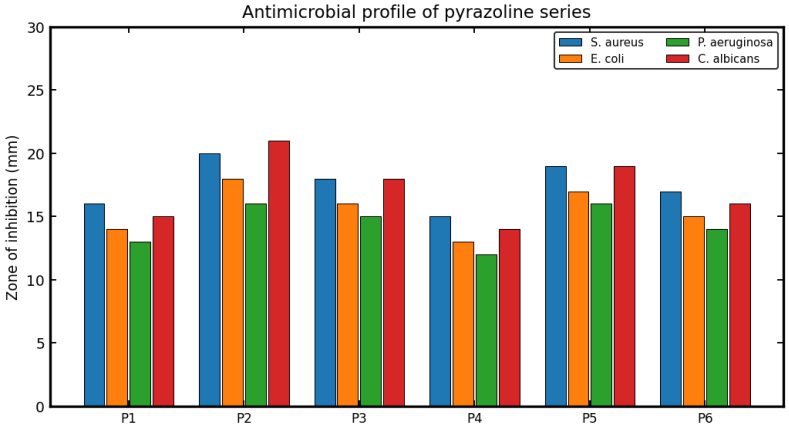


Figure 14. *Antimicrobial zones of the chalcone series (and representative heterocyclic comparison).*

4. Conclusion

Calcium acetate (10 mol %) in ethanol at 60 °C is an efficient, mild catalyst for the Claisen–Schmidt condensation, furnishing chalcone C3 in 82 % yield. Subsequent cyclisation with hydrazine hydrate produces the 2-pyrazoline P2 in 81 % yield. The original spectral plots (^{1}H NMR, ^{13}C NMR, FT-IR, ESI-MS, UV-Vis) reproduced from the thesis chapters fully confirm both structures. Biological evaluation identifies P2 as the most active member of the library across antimicrobial, anti-inflammatory and cytotoxicity assays. Pyrazoline P2 is recommended for further mechanism-of-action studies and for a focused substituent scan around the 4-chloro position.

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