Design, Synthesis and Antimicrobial Evaluation of Novel Pyrazole–Chalcone Derivatives: A Review from a Synthetic Chemistry Perspective

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Abstract
Pyrazole–chalcone derivatives are molecules in which a 1,2-diazole (pyrazole) ring is joined to an α,β-unsaturated ketone (chalcone) unit. This review describes the chemistry of these compounds: how they are designed on paper, how they are built in the laboratory, how their structures are confirmed by spectroscopy, and how simple changes in substituents alter their antimicrobial behaviour. Three reactions carry almost the whole field — Vilsmeier–Haack formylation to make the pyrazole-4-carbaldehyde, Claisen–Schmidt condensation to install the enone, and cyclocondensation with hydrazines to close the enone into a pyrazoline. Each is discussed with its reagents, conditions, mechanism and characteristic spectral signatures. Reported antimicrobial results are summarised briefly to show which structural features matter. Electron-withdrawing groups (–NO_2, –Cl, –F) on the aromatic ring attached to the olefinic carbon, and an acyl-pyridine group on the pyrazoline nitrogen, are the modifications most often associated with higher activity.

Keywords: *pyrazole; chalcone; Vilsmeier–Haack reaction; Claisen–Schmidt condensation; cyclocondensation; antimicrobial screening*

1. Introduction

Antimicrobial resistance is a growing problem, and chemists are being asked to supply new molecular skeletons rather than small modifications of old antibiotics. One inexpensive way of generating new skeletons is molecular hybridisation — covalently joining two known bioactive fragments in a single molecule so that the product carries the chemical features of both.

Pyrazole and chalcone are a convenient pair for this purpose, mainly for synthetic reasons:

1.1 Both fragments are made from cheap starting materials — acetophenones, benzaldehydes, phenylhydrazine.

1.2 The chalcone carbonyl and C=C bond provide reactive sites for further ring closure, so one intermediate gives several product families.

1.3 The pyrazole ring is thermally and chemically stable and survives the basic and acidic conditions used later in the sequence.

1.4 Substituents can be varied independently on three positions — the pyrazole N1-aryl group, the pyrazole C3-aryl group, and the aryl ring of the chalcone — so a large library is accessible from a small set of reagents.

This review deals with the chemistry of such hybrids: their design logic, preparation, characterisation and structure–activity correlations. Biological data are included only to the extent needed to explain why one substituent is preferred over another.

2. The Two Building Blocks

2.1 Pyrazole

Pyrazole is a five-membered aromatic ring containing two adjacent nitrogen atoms. N1 is pyrrole-type: its lone pair is part of the aromatic sextet, and its hydrogen (or substituent) points outward. N2 is pyridine-type: its lone pair lies in the ring plane and is available for hydrogen bonding and for protonation. The ring is π-excessive, so electrophilic substitution occurs preferentially at C4 — which is exactly the position used for formylation in the Vilsmeier–Haack reaction described below.

Positions available for variation are N1, C3, C4 and C5. In almost all antimicrobial hybrids N1 and C3 carry aryl groups, and C4 carries the carbaldehyde that becomes the chalcone linkage.

2.2 Chalcone

A chalcone is 1,3-diaryl-2-propen-1-one, Ar–CO–CH=CH–Ar'. Its chemistry is dominated by the conjugated enone:

2.2.1 The C=C bond is electron-poor because of conjugation with the carbonyl, so it behaves as a Michael acceptor towards nucleophiles such as thiols and amines.
2.2.2 Conjugation makes the molecule nearly planar and gives a strong UV absorption around 300–350 nm.
2.2.3 The 1,3-relationship between the carbonyl and the alkene allows ring closure with binucleophiles: hydrazine gives pyrazolines, hydroxylamine gives isoxazolines, urea or guanidine gives pyrimidines.
The thermodynamically favoured geometry is E, and this is what the Claisen–Schmidt reaction gives.

2.3 Why join them

In the hybrid, the pyrazole ring supplies a rigid, flat, hydrogen-bond-accepting unit, while the enone supplies electrophilic reactivity. Because the enone can also be closed into a ring, a single synthesis gives two product types from one intermediate, and the two can be compared directly.

3. Design Patterns

Reported hybrids fall into a few connectivity types, distinguished by which partner supplies the aldehyde and which supplies the ketone in the condensation step.

Type	Pyrazole component	Partner component	Product
A	4-Acetylpyrazole (ketone)	Substituted benzaldehyde	Pyrazole on the carbonyl side
B	Pyrazole-4-carbaldehyde	Aryl methyl ketone	Pyrazole on the olefin side
C	1-(Pyrazol-1-yl)propan-2-one (N–CH_2 tether)	Aromatic aldehyde	Pyrazole linked through nitrogen
D	Pyrazol-5-one bearing an acetyl group	Aromatic aldehyde	Pyrazolone-based enone
E	Formed in the last step	Chalcone + hydrazine	Chalcone cyclised to pyrazoline

Table 1. Common connectivity patterns in pyrazole–chalcone hybrids.

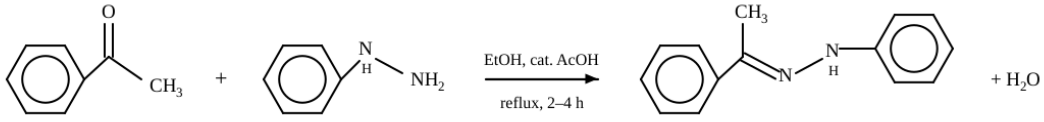
A third fragment — coumarin, barbituric acid, quinoline, adamantane or isonicotinic hydrazide — is often attached to one end. Chemically this is simply a choice of the ketone or hydrazide partner in an otherwise unchanged sequence.

4. Synthesis

The standard route is three steps. Each is reliable, uses ordinary glassware, and gives crystalline products that are purified by recrystallisation from ethanol.

4.1 Step 1 — Hydrazone formation

An aryl methyl ketone is refluxed with phenylhydrazine in ethanol with a few drops of glacial acetic acid for 2–4 hours. The acid protonates the carbonyl oxygen, the hydrazine nitrogen adds to the carbonyl carbon, and dehydration gives the hydrazone (C=N). The product usually precipitates on cooling.



Scheme 1. Acid-catalysed condensation of an aryl methyl ketone with phenylhydrazine to give the hydrazone (Step 1).

4.2 Step 2 — Vilsmeier–Haack cyclo-formylation

The hydrazone is treated with the Vilsmeier reagent, generated in situ from phosphorus oxychloride and dimethylformamide at 0–5 °C, then heated to 60–80 °C for 4–6 hours. This single operation does two things: it formylates and it closes the ring.
4.2.1 $POCl_3$ reacts with DMF to give the chloroiminium ion $[(CH_3)_2N{=}CHCl]^+$, the electrophile.
4.2.2 The enamine-like carbon of the hydrazone attacks this electrophile.
4.2.3 A second equivalent attacks the terminal methyl-derived carbon, and intramolecular attack of the NH nitrogen closes the five-membered ring.

4.2.4 Aqueous work-up on crushed ice, followed by neutralisation with sodium bicarbonate, hydrolyses the iminium salt to the aldehyde and precipitates the 1,3-disubstituted 1H-pyrazole-4-carbaldehyde.

Practical points: $POCl_3$ must be added slowly to cold DMF because the reaction is strongly exothermic; the work-up must be done on ice, since quenching a hot mixture is violent; yields are typically 60–85%.

4.3 Step 3 — Claisen–Schmidt condensation

The pyrazole-4-carbaldehyde and an aryl methyl ketone are stirred in ethanol with 40% aqueous NaOH or KOH at room temperature for 4–12 hours. Hydroxide removes an α-proton from the ketone to give an enolate; the enolate adds to the aldehyde carbonyl (aldol addition); and base-promoted loss of water (E1cb) gives the conjugated enone. Conjugation with both the carbonyl and the aryl ring makes dehydration irreversible under the reaction conditions, which is why the chalcone and not the aldol is isolated.

Common variations:

4.3.1 Sodium hydride in place of aqueous hydroxide gives shorter reaction times and cleaner products for N-tethered pyrazolyl ketones.

4.3.2 Microwave irradiation reduces reaction times from hours to minutes, with reported yields of roughly 53–94%.

4.3.3 Solvent-free thermal and ultrasound-assisted protocols avoid organic solvent altogether and are the greenest options reported for this step.

4.4 Step 4 (optional) — Cyclocondensation

Refluxing the chalcone with a hydrazine in ethanol or acetic acid closes the enone into a 4,5-dihydro-1H-pyrazole (2-pyrazoline). The terminal NH_2 adds in conjugate fashion to the β-carbon, then the second nitrogen condenses with the carbonyl and water is lost. The choice of hydrazine sets the N1 substituent:

Reagent	N1 substituent in the product	Typical conditions
Hydrazine hydrate	–H	Ethanol, reflux 4–6 h
Phenylhydrazine	$–C_6H_5$	Ethanol or AcOH, reflux 5–8 h
Isonicotinic acid hydrazide (isoniazid)	–CO–pyridin-4-yl	Ethanol/AcOH, reflux 6–10 h
Nicotinic acid hydrazide	–CO–pyridin-3-yl	Ethanol, reflux 6–8 h
Hydroxylamine hydrochloride	(gives isoxazoline, O in place of N1)	Ethanol/AcOH, reflux

Table 2. Binucleophiles used to cyclise pyrazolyl chalcones.

Oxidation of the pyrazoline — with bromine, DDQ or simply by prolonged heating in acid — gives the fully aromatic pyrazole. The three oxidation levels (open chalcone, pyrazoline, aromatic pyrazole) differ markedly in their biological behaviour, so many groups prepare all three from one chalcone.

5. Structural Characterisation

Products are identified by infrared, proton and carbon NMR, and mass spectrometry. The diagnostic signals are consistent across the literature and are the quickest way to confirm that each step has worked.

Technique	Chalcone (open enone)	Pyrazoline (cyclised)
IR	C=O 1650–1670 cm^{-1} (conjugated); C=C 1590–1610 cm^{-1}	C=O of N-acyl 1660–1680 cm^{-1}; C=N 1590–1600 cm^{-1}; no olefinic C=C
^1H NMR	Two doublets, J = 15–16 Hz (trans-olefinic Hα, Hβ); pyrazole C5–H singlet near 8.0 ppm	ABX pattern: HA and HB near 3.0–3.5 ppm (J gem ≈ 17 Hz), HX near 5.3–5.7 ppm
^{13}C NMR	C=O 188–190 ppm; olefinic carbons 120–145 ppm	C=O 165–170 ppm; C3 (C=N) ~155 ppm; C4 ~43 ppm; C5 ~60 ppm
MS	Molecular ion consistent with formula; loss of the aryl-CO fragment	Molecular ion increased by the mass of the hydrazine residue minus H_2O

Table 3. Diagnostic spectral data distinguishing chalcones from their pyrazolines.

The 15–16 Hz coupling constant in the proton spectrum is the single most useful piece of evidence: it confirms both that the enone has formed and that it is the E isomer. Its disappearance, replaced by the three-spin ABX pattern, confirms that cyclisation has taken place.

6. Antimicrobial Results in Brief

Compounds are usually screened by agar well or disc diffusion (zones of inhibition, in mm) and, in better studies, by serial broth dilution to give a minimum inhibitory concentration (MIC). The most active members of these series fall in the range 1–12.5 µg/mL; the majority are moderately active.

Compound type	Best reported result	Organisms
Isoniazid-derived pyrazolines	MIC 3.12 µg/mL	S. aureus, P. aeruginosa, C. albicans, M. tuberculosis H37Rv
Nicotinoyl pyrazolines	MIC 3.25–6.5 µg/mL	A. niger, C. albicans
Isoxazole-bearing chalcones	MIC 1 µg/mL	Bacterial panel; non-toxic to normal cells
Barbiturate-linked pyrazolyl chalcones	MIC 12.5 µg/mL	Gram-positive, Gram-negative and fungal strains
Coumarin-linked pyrazolyl chalcones	Active at 30 µg/mL	S. aureus, B. subtilis, M. tuberculosis H37Ra

Table 4. Representative activity of pyrazole–chalcone derivatives and their cyclised analogues.

A consistent observation is that activity against Gram-positive bacteria is generally stronger than against Gram-negative bacteria. The chemical explanation is the outer membrane of Gram-negative organisms, whose lipopolysaccharide layer and narrow porin channels restrict the entry of moderately lipophilic, planar molecules of this type.

7. Structure–Activity Relationships

Substituent effects reported across these series can be summarised in chemical terms.

7.1 Electron-withdrawing groups on the aryl ring attached to the β-carbon. Nitro, chloro and fluoro substituents increase activity. They lower the electron density of the C=C bond and therefore make the β-carbon a better Michael acceptor; a nitro group also adds hydrogen-bond-accepting capability and increases polar surface area.

7.2 Methoxy groups. Dimethoxy-substituted analogues are often among the best in a series despite being electron-donating, which suggests that lipophilicity and ring geometry, not electrophilicity alone, control activity.

7.3 Pyridine-carbonyl on the pyrazoline nitrogen. Pyrazolines made from isoniazid or nicotinic hydrazide consistently outperform those made from phenylhydrazine, since the added amide carbonyl and ring nitrogen provide two further hydrogen-bonding sites.

7.4 Oxidation level of the three-carbon bridge. Open chalcones tend to be the better antibacterials, pyrazolines the better antifungals, and fully aromatic pyrazoles frequently the weakest of the three. This is a direct consequence of losing the Michael-acceptor alkene on cyclisation.

7.5 Position matters more than count. Para- and meta-substituted analogues usually differ substantially, so each isomer must be made and tested rather than inferred.

8. Limitations and Scope for Further Work

8.1 Many reports give only zones of inhibition. These depend on diffusion rate as well as on potency and cannot be compared between laboratories; MIC values from standardised broth dilution should be the minimum.

8.2 Since the enone is an electrophile, apparent activity may reflect general reactivity with thiols rather than a specific interaction. A cytotoxicity test on a normal mammalian cell line should accompany every screen.

8.3 Solubility, partition coefficient and stability data are rarely measured, although they explain much of the variation in observed activity.

8.4 Green methods — microwave, ultrasound, solvent-free and aqueous conditions — work well in this chemistry but are still used by a minority of groups.
8.5 Matched sets (the same substitution pattern as chalcone, pyrazoline and pyrazole) are the most informative experiment available in this area and are easy to prepare, yet are seldom reported.

9. Conclusion

Pyrazole–chalcone derivatives are built by a short, robust sequence — hydrazone formation, Vilsmeier–Haack cyclo-formylation, Claisen–Schmidt condensation, and optional cyclocondensation with a hydrazine. Every step uses inexpensive reagents and gives crystalline products that are easily characterised, which is why the field has produced so many analogues. The chemistry responsible for their antimicrobial behaviour centres on two features: the electrophilic α,β-unsaturated ketone and the hydrogen-bonding capacity of the nitrogen heterocycles. Electron-withdrawing substituents on the olefinic aryl ring and an acyl-pyridine group on the pyrazoline nitrogen are the modifications most reliably associated with improved activity. Future work in this area would benefit less from new analogues than from standardised measurement — MIC determinations, cytotoxicity controls and matched comparisons across the three oxidation levels of the central bridge.

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