

Review Article

Ultrasound-Assisted Synthesis of Pyrazole–1,3,4-Oxadiazole Hybrids: Synthetic Advances, Antimicrobial Activity, and Structure–Activity Relationships

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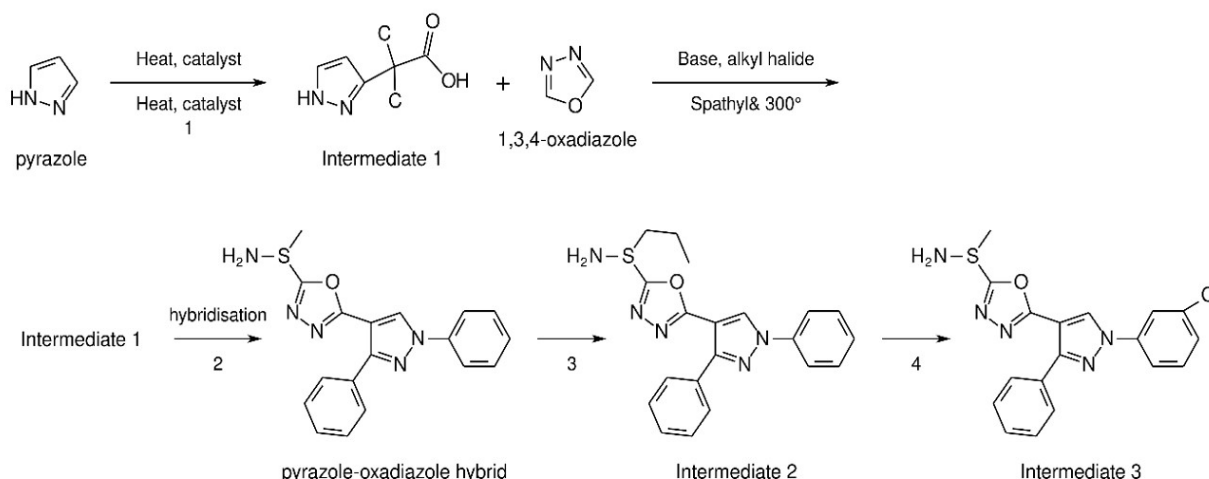
Abstract

Pyrazole–1,3,4-oxadiazole hybrids are the combination of two privileged heterocyclic pharmacophores with potential antimicrobial significance and the ultrasound-assisted synthesis is a potentially efficient method for the construction of heterocycle. This is a critical review of the evidence linking these two research directions. The physicochemical aspects of acoustic cavitation and the reported sonochemical effects on azole synthesis are first considered, and then direct and adjacent evidence for systems of pyrazole–1,3,4-oxadiazole is compared. The antimicrobial activity against Gram-positive and Gram-negative bacteria, mycobacteria and fungi is evaluated, with particular attention to the comparability of assays and structure–activity relationships. A significant gap in the literature exists in terms of conducting simultaneous studies in the design of pyrazole–1,3,4-oxadiazole hybrid compounds, their synthesis using ultrasound and their evaluation of antimicrobial activity: relatively small number of studies meet this criteria while several good biological activities are found using conventional or microwave-assisted synthesis. Therefore, sonochemistry is not yet considered as a proven source of biological benefits for this chemotype. Also, the data is limited by heterogeneous susceptibility methods, incompleteness of the acoustic parameters, incomplete mechanistic validation and lack of cytotoxicity, resistance and in vivo data. Standardized antimicrobial assays, complete sonochemical parameterization, thermal and microwave control and integrated selectivity and mechanism studies are recommended.

1. Introduction

AMR is now being referred to as a “slow-moving pandemic” and there are few truly new chemotypes of antibacterial in the clinical pipeline. In medicinal chemistry two answers have been forthcoming. The first approach involves linking two pharmacophoric fragments showing complementary or augmenting activities to broaden the spectrum and/or enhance the efficacy or overcome the resistance in a single molecule [1]. The second is the use of green and enabling synthetic technologies, such as microwave, mechanochemistry and ultrasound which shorten the development time and minimize the impact of the medicinal-chemistry campaign on the environment [2, 3].

Pyrazole and 1,3,4-oxadiazole ring are two well known “privileged scaffolds”. The pyrazole backbone is found in many marketed drugs, and has an extremely broad spectrum of bioactivity (antibacterial, antifungal) for a variety of substituents. The 1,3,4-oxadiazole ring is a bioisostere of ester and amide, is metabolically stable, is a good hydrogen bond accepting group, and has been reported to be active against



Scheme 1: The molecular-hybridization concept: fusion of the pyrazole and 1,3,4-oxadiazole privileged scaffolds into a single pyrazole–oxadiazole hybrid pharmacophore

several drug-resistant organisms like methicillin resistant *Staphylococcus aureus* (MRSA) and several reviews are reported on the activity of 1,3,4-oxadiazole ring [4, 5]. Hence, the combination of both to afford pyrazole–oxadiazole hybrids is a natural design and a steady flow of such hybrids with antitubercular and broad-spectrum antimicrobial activity have come up [6, 7].

In this medicinal-chemistry background, the ultrasound-assisted (sonochemical) synthesis has been found to be a desired pathway to the synthesis of azole heterocycles. This is indeed correct: many hours of reflux and normal heating for completion of the cyclodehydration and condensation can be achieved in a few minutes with clean reaction profiles and yields of isolated products are higher when using sonication [2, 8, 9]. The aim of this review is not only to put a positive light on this claim, but to challenge it as well with the available data specifically for pyrazole–oxadiazole hybrids, and to pose a skeptical question: how much of the attractive antimicrobial data for the pyrazole–oxadiazole hybrids was really achieved by ultrasound-assisted chemistry and how valid is the underlying SAR?

2. Ultrasound-Assisted Synthesis: Principles and Relevance

2.1. Acoustic cavitation as the driving force

The chemical effects of ultrasound in the frequency range of 20 kHz – 1 MHz are not directly on the molecular vibrational/electronic level but indirectly and mechanically through acoustic cavitation [2]. The compression and rarefaction cycles alternate, causing the formation, growth and violent collapse of micrometer-scale bubbles in the liquid. This implosive collapse results in local transient “hot-spots” with extreme conditions (temperatures of the order of 4500-5,000 K, and pressures of up to 1000 atm), with strong micro-mixing and shock waves, but with the bulk medium near ambient temperature [2, 3, 10]. This combination is responsible for the sonochemical signature that involves reducing effective activation barriers, improving mass transfer, increasing selectivity and, in a heterogeneous system, continually fragmenting and cleaning the catalyst surface as shown in Figure 1.

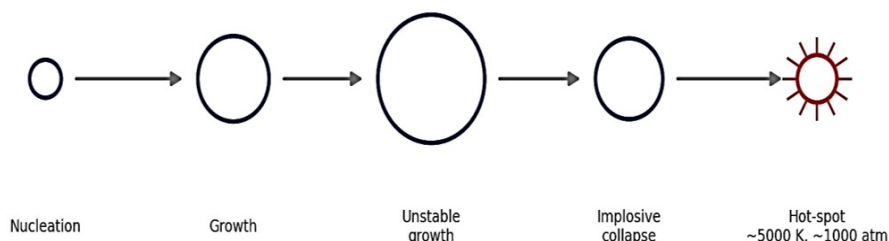


Figure 1: The acoustic-cavitation life-cycle. Bubbles nucleate, grow over successive rarefaction cycles, become unstable and collapse implosively, producing transient hot-spots while the bulk liquid stays near ambient temperature

These collapse events can be modeled and approximated by an adiabatic (Nolting–Neppiras-type) model of the form given in Equation (1), with $T_{\max}$ a scaled peak hot-spot temperature, P_m/P a pressure ratio at the time of collapse and γ the polytropic index. The radius of a bubble which resonates with a driving field at frequency f_r is given by the so called ‘Minnaert relation’ as shown in Equation (2) in which R_0 is the equilibrium radius, p_0 is the ambient pressure and ρ is the liquid density:

The first of these relationships is the chemical “punch” of each collapse Equation (1) and the second is the number of bubbles that can be excited by a given frequency of the transducer Equation (2). They are also aware of the important solvent effects on the efficiency of a sonochemical reaction because of the physical properties of the solvent, and the need for the acoustic parameters to be carefully described, such that the results can be reproduced.

$$T_{(\max)} = T_0(P_{m/p})(\gamma - 1) \quad (1)$$

$$f_r = \frac{1}{2\pi R_0} \sqrt{\frac{3\gamma P_0}{\rho}} \quad (2)$$

Several of the principles of green chemistry are being applied, such as the use of less hazardous and less quantity of solvents, shorter reaction time and energy saving [2, 3]. Recently representative reviews have been published that demonstrate the use of sonochemical protocols in combination to prepare N- and O-azoles and other bioactive heterocycles amongst which the most sonication beneficial ring-closing reactions [2, 3, 11].

2.2. Documented gains for azole ring construction

Two single gains can be recorded with the use of azole ring construction:

There is good agreement in the quantitative aspects of ultrasound in azole chemistry as judged from the results obtained by different laboratories. Characteristic figures for the previous five years (and a few important earlier reports) have been collected in Table 1. The trend has been that the reaction time decreases from hours to a few minutes and the isolated yield increases, frequently in the absence of a catalyst or solvent [12] as shown in Figure 2.

The acceleration is in agreement with the Arrhenius relationship Equation (3) that a higher virtual ‘effective local temperature’ (T) due to better mixing and a higher rate constant (k) for a given activation energy (E_a) would lead to a higher acceleration.

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (3)$$

Table 1: Representative ultrasound-assisted syntheses of oxadiazole/pyrazole-containing scaffolds, illustrating the typical time-and-yield advantage over conventional heating. Note that only [8, 13] involve a pyrazole ring directly fused or linked to an azole partner; the remainder are mono-azole or adjacent hybrids

Scaffold / transformation	Conditions	Time	Yield	Ref.
1,3,4-Thiadiazole-pyrazolyl-thiazolidin-4-one hybrids (multicomponent)	EtOH, ultrasound, 50°C	55-65 min	91-97%	[8]
5-Substituted 1,3,4-oxadiazole-2-thiols	Ultrasound, low-solvent, acid/base-free (CS ₂ , drops of DMF)	Short	Good-excellent	[14]
1,3,4-Oxadiazole-2(3H)-thione Mannich bases (antifungal)	Ultrasound + molecular sieves	vs 15-20 h reflux	78-90%	[15]
Indole-oxadiazole-triazole hybrids	Ultrasound vs conventional/microwave	15-35 min	82-93%	[16]
1,3-Diphenylpyrazole spirooxindolo-1,2,4-oxadiazoles	Base-mediated, one-pot, ultrasound (green)	Reduced	Good	[13]

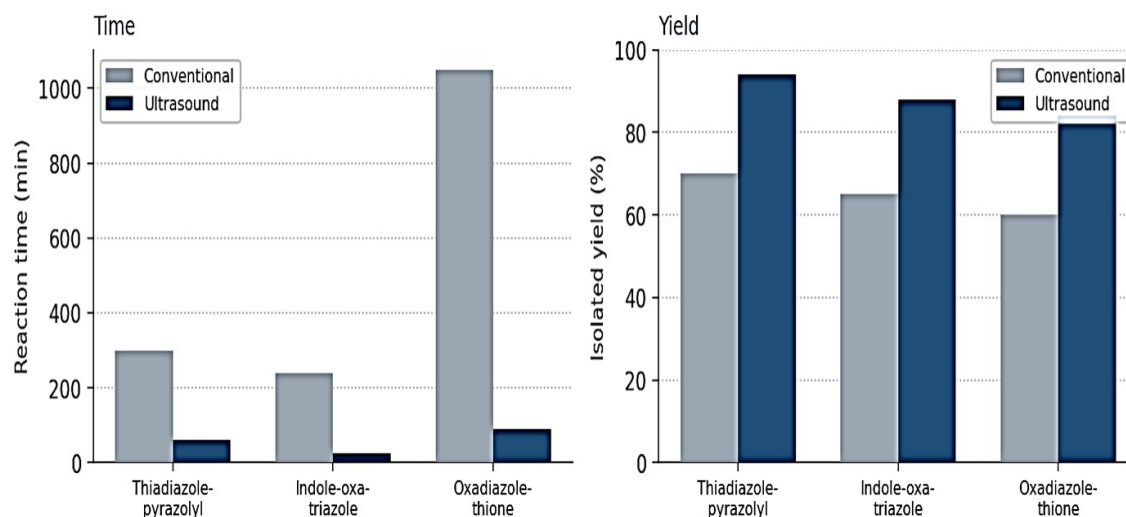


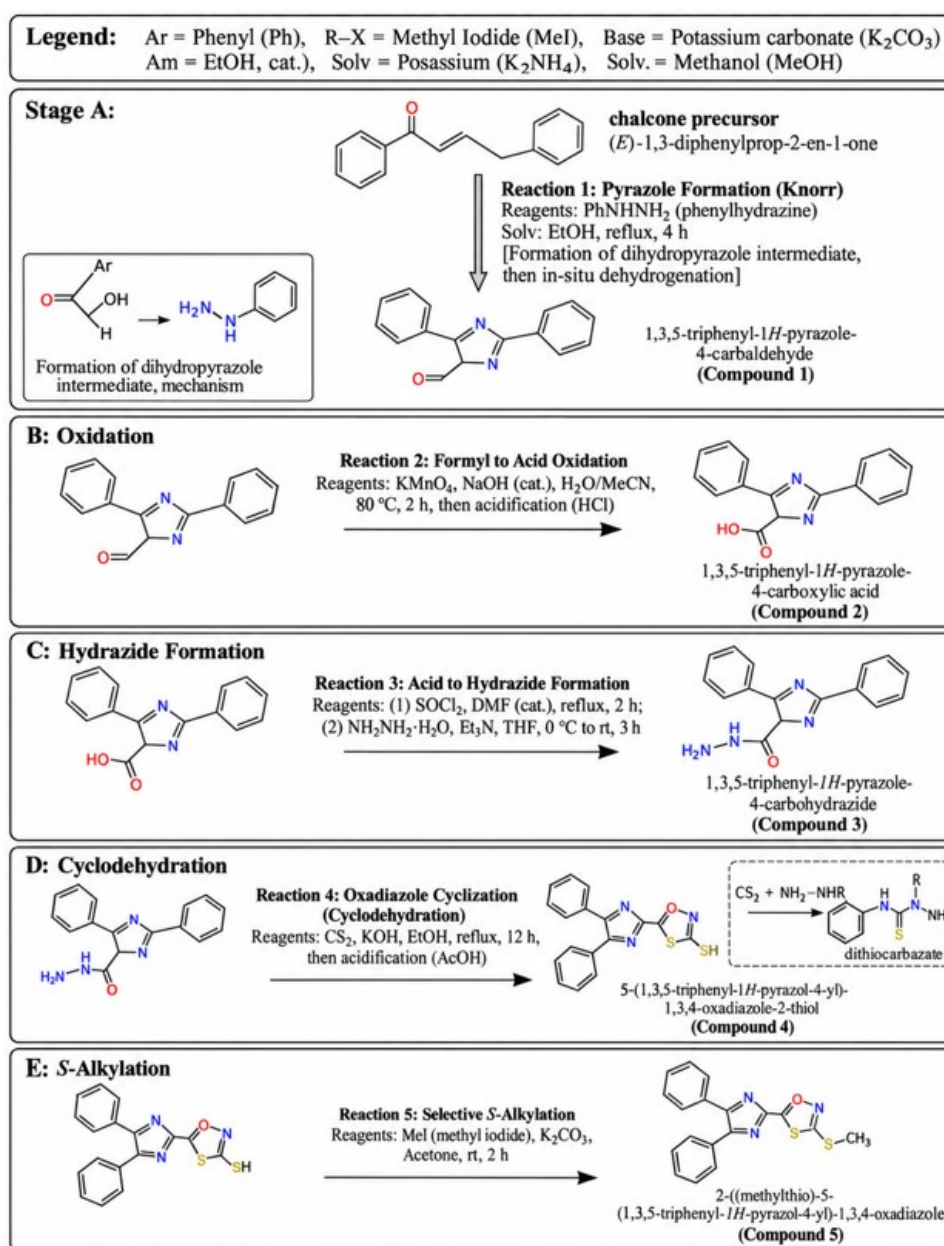
Figure 2: Illustrative comparison of reaction time and isolated yield for ultrasound versus conventional heating across representative azole syntheses (data adapted from refs [8, 15, 16]). The qualitative pattern — shorter times and higher yields under sonication — is robust, but absolute values depend strongly on the (often under-reported) acoustic conditions

However, neat sonication is not enough and magnetic ferrites, chitosan Schiff bases, MOF based nano-polymers, and other catalysts which are recyclable are used in combination with ultrasound and several of these systems are found to catalyze the formation of azole and fused pyrazole [17, 18].

At this level of description, there are two cautions that must be issued. First, the acoustic parameters affecting the reproducibility (frequency, calibrated power or acoustic intensity, probe-versus-bath geometry, duty cycle and temperature control) are not always reported and this makes it often impossible to compare “ultrasound conditions” between different studies. Secondly, very few comparisons are available between the use of microwave irradiation and sonication and even if there are, few factors other than equipment availability will dictate choice between them [16].

3. Synthetic Strategies for Pyrazole–Oxadiazole Hybrids

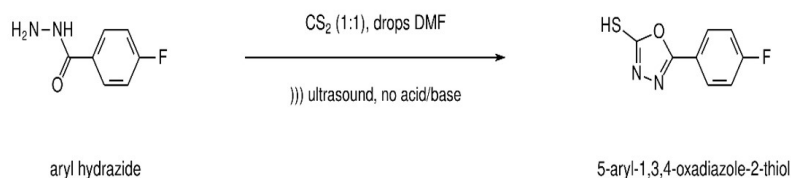
The two rings are joined together in sequence by the predominant disconnection. A pyrazole with a carboxylic acid, ester or carbohydrazide handle (usually from a Vilsmeier–Haack or Knorr-type chemistry, 1-aryl-3-aryl-1H-pyrazole-4-carbaldehyde or -4-carboxylic acid) is elaborated into the aroyl- or acyl-hydrazide which is then oxidatively or dehydratively cyclised to the 1,3,4-oxadiazole. The most frequently used ring closing reagents and conditions include carbon disulphide/KOH (2-thiol/2-thione oxadiazoles), aryl/acyl chlorides and dehydrating agents, and oxidative cyclisation of N-acylhydrazones [4, 6, 14]. The 2-position of the oxadiazole is then frequently modified, e.g. S-alkylation of the thiol, Mannich aminomethylation or amide formation or Schiff-base formation to produce libraries [15].



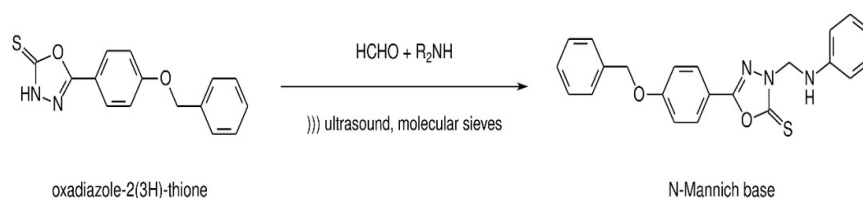
Scheme 2: Representative linear route to pyrazole–1,3,4-oxadiazole hybrids: Knorr-type construction of a 1,3-diaryl-pyrazole-4-carbaldehyde (1), oxidation to the 4-carboxylic acid (2), conversion to the carbohydrazide (3), CS_2/KOH cyclodehydration to the oxadiazole-2-thiol (4), and S-alkylation to the functionalized hybrid (5). Structures are generic exemplars

It is the cyclodehydration and condensation steps in this sequence, which are most susceptible to sonication; that is why ultrasound

has been used successfully on the oxadiazole-forming step in neighboring systems [14–16]. One of the more recent examples of the use of ultrasound to assemble a new spirooxindolo-1,2,4-oxadiazole hybrid with a pyrazole motif is mediated by a base [13].



Scheme 3: Sonochemical, low-solvent, acid/base-free formation of a 5-aryl-1,3,4-oxadiazole-2-thiol from an aryl hydrazide and CS₂ (after ref [14]). The cyclodehydration step is the one most accelerated by cavitation



Scheme 4: Ultrasound- and molecular-sieve-assisted N-Mannich functionalization of a 1,3,4-oxadiazole-2(3H)-thione (after ref [15]), a representative late-stage diversification used to build antimicrobial libraries

3.1. A candid assessment of the direct evidence

The evidence base should be separated into direct and supporting evidence. Studies satisfying all three criteria—pyrazole–1,3,4-oxadiazole hybrid, ultrasound-assisted preparation, and antimicrobial evaluation—remain limited. Several important antimicrobial pyrazole–oxadiazole series were prepared by conventional or microwave-assisted methods, whereas sonochemical literature more often concerns related pyrazole-containing azoles or oxadiazole derivatives.

The original reaction equations and schemes are retained throughout this revised manuscript because they document the actual synthetic routes discussed in the literature. For interpretation, the evidence is classified as: Tier 1, direct target evidence; Tier 2, near-neighbor sonochemical evidence; and Tier 3, conventional or microwave target-scaffold evidence used for biological and SAR context.

4. Antimicrobial Potential

It has been reported in recent literature that pyrazole–oxadiazole and related azole hybrids are active against most of the clinically important organisms like Gram positive cocci (*S. aureus* including MRSA, *Enterococcus faecalis*, *Streptococcus pyogenes* and *Bacillus subtilis*), Gram negative rods (*Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella* spp.), *Mycobacterium tuberculosis* and fungi (*Candida albicans*, *Candida glabrata*, *Cryptococcus neoformans*, *Aspergillus* spp.) [4, 5, 19, 20]. Some of them (mainly *P. aeruginosa* and *A. baumannii*) are members of the multidrug resistant ESKAPE group and the chemotype is therefore of clear strategic importance in the hospital environment.

4.1. Antibacterial activity

The members of this class range from quite potent (low micromolar potency) to not so brilliant. In fact, pyrrole-ligated 1,3,4-oxadiazoles with indophenol-side chain showed an excellent activity against the hard-to-kill gram-negative bacterium *A. baumannii* with MIC value $< 2 \mu\text{g/mL}$ which is an outstanding result for an intrinsically resistant bacterium [19]. In this regard, the MBC of 4-nitro and 3-nitro derivatives of 1,3,4-thiadiazole-pyrazolyl-thiazolidin-4-one hybrids, synthesized under ultrasound showed 4- and 2- fold higher activity than the activity shown by ciprofloxacin against *P. aeruginosa* [8]. Dihydro-1,3,4-oxadiazoles based on pyridine-pyrazole also provided compounds with interesting antibacterial and antifungal profile with the aid of DFT and docking studies [20]. In the agrochemical field, 1,3,4-oxadiazole sulfonamides bearing a pyrazole group could inhibit *Xanthomonas oryzae* pv. *oryzae* completely at 100 mg/L, even more effective than the commercial bactericides; the reminder to the chemotype value is not limited to human pathogens [5]. Besides, similar to clinical range, 2-(pyrazol-4-yl)-1,3,4-oxadiazole with imidazole fragment were proved to exhibit activity on the bacterial cell membrane and mechanism studies (growth rate, SEM and biofilm assays) confirmed the docking-based rationale.

4.2. Antitubercular activity

One combination that is found to be very effective against *M. tuberculosis* is pyrazole–oxadiazole. Several pyrazole–oxadiazole hybrids were synthesized by microwave assisted synthesis [6] and the molecules were rationalized via docking in the active site of enoyl-ACP reductase (InhA) which is shown to be highly active against *M. tuberculosis* H37Ra and *M. bovis* BCG. In the latter, it was suggested that pyrazole linked 1,3,4 oxadiazole hybrids offered compounds with MICs as low as 1.56 $\mu\text{g/mL}$ against *M. tuberculosis* H37Rv similar to the MICs of ethambutol [7]. Some of the spirooxindolo-1,2,4-oxadiazoles derived from 1,3-diphenylpyrazole were found to exhibit antitubercular activity at their MIC values of 3.125 $\mu\text{g/mL}$ against H37Rv, which shows that the sonochemical route could be used to obtain antitubercular hits in the scaffold family of the compounds [13].

4.3. Antifungal activity

Reports are less complete and there are positive indications of antifungal screening. Ultrasound and molecular-sieve based 1,3,4-oxadiazole-2(3H)-thione derivatives have been evaluated for their antifungal activity on a set of seven fungal strains including *C. albicans*, *C. glabrata*, *C. neoformans* and *Aspergillus* species and gave promising antifungal templates [15]. The sonochemically synthesized 5-aryl-1,3,4-oxadiazole-2-thiols were also found to be active against bacterial and fungal strains, as the 4-fluorophenyl derivative was found to be active against both the bacterial and fungal strains [14]. Selected pyrazole–thiazole hybrids were active against *C. albicans* when compared to griseofulvin [9] and neighboring pyrazole–thiazole hybrids offered further antifungal leads. One of the rare series in this field to show a true selectivity window is that of oxadiazole–thiadiazole hybrids, which had MIC 0.78–3.12 $\mu\text{g/mL}$ against four species of *Candida*, and MTT assay showed that they were not toxic at MIC concentrations [21].

4.4. Synthesis of reported activity

As shown in Figure 3, the selected hybrids exhibit target-specific antimicrobial potency compared with reference drugs, with lower MIC values indicating greater potency.

Table 2: Snapshot of recent antimicrobial findings for pyrazole–oxadiazole and adjacent azole hybrids. Method column makes explicit the recurring observation of this review: the most potent hits are split between ultrasound, microwave and conventional routes rather than dominated by sonochemistry

Hybrid / scaffold	Key organisms	Reported potency	Method	Ref.
Iodophenol pyrrole–1,3,4-oxadiazole	<i>A. baumannii</i>	MIC < 2 $\mu\text{g/mL}$	Conventional	[19]
Thiadiazole–pyrazolyl–thiazolidinone	<i>P. aeruginosa</i>	2–4 $\times$ > ciprofloxacin (MBC)	Ultrasound	[8]
Pyrazole-linked 1,3,4-oxadiazole	<i>M. tuberculosis</i> H37Rv	MIC 1.56 $\mu\text{g/mL}$ ($\approx$ ethambutol)	Conventional/design	[7]
Microwave pyrazole–oxadiazole	<i>M. tuberculosis</i> / <i>M. bovis</i>	Significant (InhA docking)	Microwave	[6]
Pyrazole spirooxindolo-oxadiazole	<i>M. tuberculosis</i> H37Rv	MIC 3.125 $\mu\text{g/mL}$	Ultrasound	[13]
5-Aryl-1,3,4-oxadiazole-2-thiol	Broad bacterial + fungal	Broad-spectrum (4-F best)	Ultrasound	[14]
Oxadiazole-2(3H)-thione Mannich	<i>Candida</i> / <i>Aspergillus</i> spp.	Promising antifungal	Ultrasound + sieves	[15]

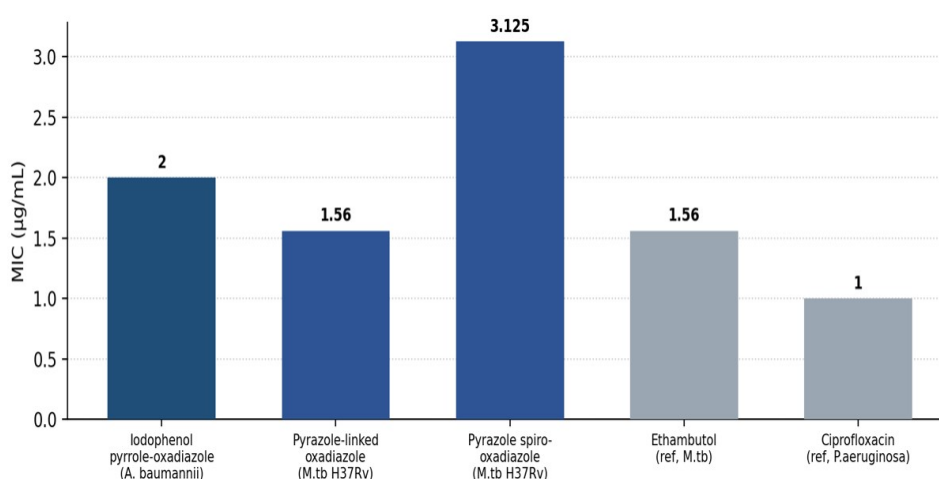


Figure 3: Minimum inhibitory concentrations of selected potent hybrids against their respective target organisms, shown alongside reference drugs (lower bars indicate greater potency). Because the bars refer to different organisms, the figure illustrates per-target potency rather than a single head-to-head ranking

5. Structure–Activity Relationships

Notwithstanding the heterogeneity of assays and series, several SAR themes recur with enough consistency to be useful for design — alongside at least one instructive contradiction that should temper any claim of a universal rule as shown in Figure 4.

Comprehensive Structure-Activity Relationship (SAR) of the Hybrid Library: A Critical Review of Emerging Evidence

A green route to new antimicrobials—and a candid view of how far the evidence currently goes

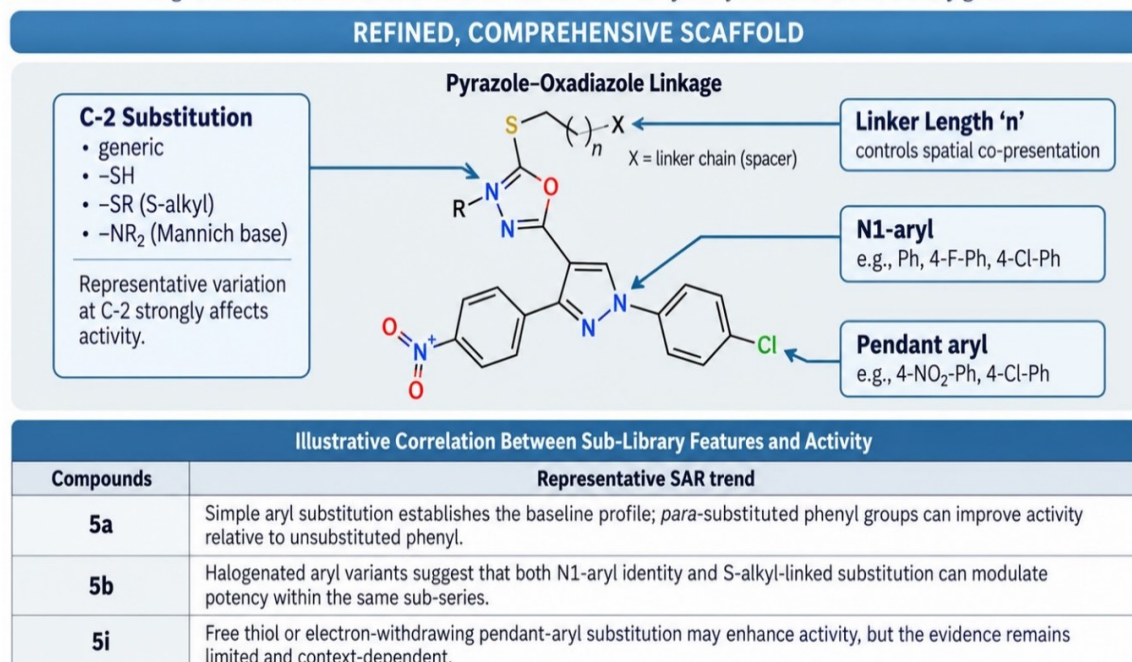


Figure 4: Composite structure-activity map for the pyrazole-oxadiazole chemotype, locating the four design handles discussed in the text: the oxadiazole C-2 substituent, the inter-ring linkage, the electron-poor/halogenated pendant aryl, and the pyrazole N1-aryl group. Trends are general tendencies, not target-independent rules (see Section 5.2). The map also makes the review's verification convention explicit: the "verified" marker flags the precision sub-library whose trends are anchored in specific, primary-literature compounds (the "verified" data of Table 2), whereas the limitations banner flags the broader SAR claims that still rest on small, non-standardized samples (the "general / illustrative" category of Table 3 and the data-provenance note of Section 6). Reading the figure and the tables together is intended to keep the visual and the text a single, internally consistent fabric.

5.1. The oxadiazole 2-position is a primary activity lever

It is the C-2 substitution on the 1,3,4-oxadiazole that again and again proves to be critical. Modulation of potency and spectrum is seen with thiol/thione functionality, S-alkyl or S-aryl groups and elaboration at this position by aminomethyl (Mannich) or Schiff base bonds [14, 15]. The oxadiazole oxygen and nitrogen are capable of hydrogen-bonding and changes in these regions affect target binding and lipophilicity, which influences membrane permeation, particularly important for Gram-negative bacteria which have a highly restricted outer membrane.

5.2. Pendant aryl substitution: the (qualified) dominance of electron-withdrawing and halogen groups

Substituents with the pendant aryl rings that are more electron withdrawing such as nitro groups and halogens have a tendency to increase potency for antibacterial activity. Among the most active compounds are the indophenols, the indophenol oxadiazole and the nitro substituted thiadiazole-pyrazolyl-thiazolidinones which are included in several series and the importance of heavy halogens is apparent, most likely due to the combination of the increased lipophilicity, the halogen bonding and the changed electron density [19]. The site of substitution is important, too, as *para* substitution is often best and *ortho* substitution is sometimes sterically unfavorable.

This is not a rule and the exception is recommended. For antitubercular pyrazole linked oxadiazoles, it was the *para* substituents (-OH, -NH₂) which showed the good activity against *M. tuberculosis* H37Rv [7]. The difference between the two should not be seen as a clash of results but as a demonstration that optimal electronics for disruption of the Gram-negative envelope or for a specific bacterial enzyme do not need to be optimal for disruption of the mycobacterial target, InhA. A review that will be provided by EWG will therefore be over general, that is, it will simply talk about "EWG is better".

5.3. Pyrazole substitution and the role of the linker

On the pyrazole side, the N-1 aryl group and the C-3 substituent influence the electronic nature of the ring as well as overall lipophilicity and in some cases bulky groups on the aryl ring adjacent to the heterocyclic junction aid and in others hinder activity (as seen in pyrazole-thiazole series and trifluoro methylated pyrazole hybrids [9, 22]). Another and less studied variable to control the conformational freedom and relative orientation of the two pharmacophores is the linker between two rings, pyrazole and oxadiazole (direct bond, methylene, carbonyl, thioether or fused or spiro junction).

5.4. Physicochemical framing

In general, the more effective antimicrobial agents fall within a "just right" lipophilicity window where the agent is not lipophilic enough to be insoluble/membrane-nonpenetrating and not too hydrophilic to be toxic to membranes. Generally, the more drug-like the more promising biological hits (reported in [7, 13, 20]) if an ADME/Lipinski has been reported in silico. Although a useful design compass, this is not to be

read as a sign of a well-established mechanism.

6. Structural Confirmation and Structure–Property Correlation

The chemical equations and schemes in this section are retained because they are part of the evidence base and show the transformations by which the reported heterocyclic frameworks were constructed. Structural assignment should nevertheless rely on orthogonal analytical evidence.

FT-IR, ^1H -NMR, ^{13}C -NMR, HRMS, elemental analysis and, where available, X-ray diffraction provide complementary levels of structural support. FT-IR can support functional-group changes but should not be treated as definitive proof of ring connectivity. HRMS supports molecular-formula assignment, whereas complete NMR assignments provide stronger evidence for connectivity. Single-crystal X-ray diffraction, when available, offers the most direct structural confirmation.

Generalized spectral ranges may be useful as explanatory context, but they must remain clearly distinguished from compound-specific experimental data. Likewise, docking and related computational approaches should be interpreted as hypothesis-generating rather than as direct proof of antimicrobial mechanism or potency.

Table 3: General / illustrative Category

Technique	Diagnostic signal	Typical range / value	What it confirms
FT-IR	Loss of hydrazide N–H / C=O; new ring C=N	N–H 3150–3300; C=O 1640–1690; C=N 1600–1640 cm^{-1}	Oxadiazole ring closure
FT-IR	=C–O–C= ether stretches	1240–1270 and 1020–1090 cm^{-1}	1,3,4-oxadiazole core
FT-IR	C=S (thione); NO ₂ (nitro R)	C=S 1150–1280; NO ₂ 1500–1560 / 1320–1360 cm^{-1}	C-2 thione; R substituent
^1H -NMR	Pyrazole C–H singlet; S–CH ₂ singlet	δ 7.8–8.5; δ 4.3–4.7 ppm	Connectivity / S-alkylation
^1H -NMR	Trans-vinyl / aryl couplings	^3J 16 Hz (E); ^3J 8–9 Hz (para-AA ‘BB’)	Linker configuration / substitution
^{13}C -NMR	Oxadiazole C-2/C-5; C=S; C–F	δ 163–168; 178–185; $^1J_{\text{CF}}$ 245–250 Hz	Ring identity / fluorine
HRMS	[M+H] ⁺ vs calculated; M+2 isotope	≤ 5 ppm; Cl $\approx$ 3:1, Br $\approx$ 1:1 M/M+2	Molecular formula / halogen count
Elem. anal.	C, H, N (S) found vs calc.	within $\pm 0.4\%$	Identity and purity
Melting pt.	Sharp range; rise on cyclisation	$\approx 1\text{--}2^\circ\text{C}$ span	Purity; hybrid formation (indirect)
Docking	Binding energy / pose (intra-series)	More negative score $\leftrightarrow$ lower MIC/IC ₅₀	Mechanistic hypothesis only

7. Critical Appraisal and Limitations

A review to only state positive outcomes would be a disservice to the field. At the moment, there are various systematic drawbacks which limit the conclusions that can be drawn.

Lack of on target studies. Considering the above discussion in Section 3.1, there are very limited reports that fulfill the above three criteria, i.e., pyrazole–oxadiazole hybrid, ultrasound preparation and microbial evaluation. Most of the supporting evidence comes from neighboring scaffolds or is adapted from microwave/conventional preparations and so claims unique to ultrasound-assisted pyrazole–oxadiazole preparations are based on limited evidence.

- Non-standardized susceptibility testing. Several assays are used (agar diffusion, broth microdilution, MIC, MBC, endpoint of zone of inhibition), several inoculum sizes are used and several sets of reference drugs are used (ciprofloxacin, amoxicillin, chloramphenicol, ethambutol, fluconazole, griseofulvin). It is difficult to compare cross-study and perform metanalysis due to different protocols (CLSI/EUCAST) used to adhere to guidelines.
- Errors in the units and reporting. Not all primary literature data are tabulated and the units of concentration are not always specified in a consistent way in the primary literature (e.g., $\mu\text{g/L}$ instead of $\mu\text{g/mL}$). Reviewers/readers are encouraged to use the units in the context of the assay - rather than just believe the potencies that are claimed in the head-line.
- Under-reported acoustic parameters. The number of times that the sonochemical procedures are not reported in terms of frequency, calibrated power/intensity, probe versus bath configuration, duty cycle, and bulk-temperature control makes reproducing the procedures and determining the “green” advantage of sonochemistry over a properly optimized thermal or microwave control difficult.
- Use of docking as mechanism. Rationalization of activity is normally done by molecular docking (e.g. InhA or DNA gyrase) and sometimes by enzyme-inhibition assays and/or mutational validation or resistance mapping. A docking pose is a hypothesis; it is NOT a mode of action.

Information on selectivity, toxicity and resistance is scarce. Cytotoxicity against mammalian cells, selectivity indices, time–kill kinetics, studies of biofilm activity, and resistance induction are seldom reported and efficacy in vivo is seldom reported. These are required to evaluate the translational potential and potency may be due to general toxicity and not antimicrobial selectivity.

Publication bias and series bias. Libraries are likely to be small, and focus on a single favorable core, while the negative results are under-reported, which can result in an over-stating of the reliability of an SAR trend.

One low-cost, simple way of protecting against some of these issues is to provide a selectivity index with each potency. The selectivity index (SI) is the ratio of the agent’s cytotoxicity on mammalian cells (CC₅₀) and the agent’s antimicrobial activity (MIC); a value of $> \sim 10$ was used as a provisional threshold value to discriminate between selective antimicrobial activity and general cytotoxicity. Of these

pyrazole/oxadiazole studies, only those that include both MIC and cytotoxic activity (such as the nano catalyst derived pyrazole where the cytotoxic activity has been explicitly worked out and the low MIC, nontoxic, anticandidal oxadiazole/ thiadiazol hybrids [17, 21]) indicate that the biology may be interpreted.

$$SI = \frac{CC_{50}}{MIC} \quad (4)$$

8. Conclusions and Future Perspectives

The available evidence supports pyrazole–1,3,4-oxadiazole frameworks as promising antimicrobial chemotypes, but it does not yet establish that ultrasound-assisted synthesis is responsible for superior antimicrobial performance of this specific hybrid class. Direct studies combining the target scaffold, ultrasound synthesis, and standardized antimicrobial evaluation remain limited.

Future work should report frequency, acoustic power or intensity where available, probe or bath configuration, duty cycle, reaction volume, solvent composition, and bulk-temperature control. Sono chemical protocols should be compared with appropriately optimized conventional thermal controls and, where relevant, microwave controls so that any observed advantage can be attributed to the irradiation method.

Article Information

Ethical Approval: Not Applicable.

Informed Consent: Written informed consent was obtained from all participants before their inclusion in the study.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Author Contributions: All authors contributed to the conception and design of the study, data collection/analysis, interpretation of results, and preparation and approval of the final manuscript.

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