



Synthesis, spectral characterization and antimicrobial evaluation of novel 2-(4-methoxyphenyl)-5-aryl-1,3,4-oxadiazoles: In vitro screening, molecular docking against DNA gyrase B and CYP51, and in silico ADME profiling

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ABSTRACT

Ten 2-(4-methoxyphenyl)-5-aryl-1,3,4-oxadiazoles (4a to 4j) were prepared from methyl 4-methoxybenzoate through the acid hydrazide and N,N'-diacylhydrazine intermediates, using phosphoryl chloride as cyclodehydrating agent, in 68 to 85 per cent yield. Structures were assigned from FT-IR, proton and carbon nuclear magnetic resonance and mass spectrometric data, loss of the carbonyl and N-H stretches together with a new band between 1604 and 1619 cm⁻¹ confirming ring closure in every case. Activity was measured by broth microdilution against two Gram-positive bacteria, two Gram-negative bacteria and two fungi. The 2,4-dichlorophenyl derivative 4j and the 4-chlorophenyl derivative 4c were the most active members, giving minimum inhibitory concentrations of 3.90 and 7.81 microgram per millilitre against Staphylococcus aureus. Compound 4j reduced viable counts by 3.29 log units within 8 h at four times its minimum inhibitory concentration. Docking against Escherichia coli DNA gyrase B (PDB 1KZN) and sterol 14 alpha-demethylase (PDB 1EA1) gave affinities between -6.6 and -9.4 kcal per mole, which correlated with observed potency (R² = 0.899). All ten compounds satisfied Lipinski criteria without violation, and halogenated derivatives are the more promising leads.

Keywords: 1,3,4-Oxadiazole; antimicrobial activity; minimum inhibitory concentration; molecular docking; DNA gyrase B; structure-activity relationship

1. INTRODUCTION

Bacterial resistance now accounts for a substantial share of global mortality, an analysis covering 204 countries attributing 1.27 million deaths directly to resistant bacterial infections during 2019, with Staphylococcus aureus and Escherichia coli carrying much of that burden (Antimicrobial Resistance Collaborators, 2022). Fungal infection follows a similar pattern, since prolonged azole therapy selects for Candida strains that respond poorly to fluconazole. The gap between the spread of resistance and the arrival of new scaffolds has renewed interest in small heterocycles that can be assembled quickly and modified at several positions. The 1,3,4-oxadiazole ring is one such scaffold. It serves as a bioisostere for esters and amides because it resists hydrolysis while retaining hydrogen-bond acceptor character, and the 1,3,4-isomer carries roughly an order of magnitude lower lipophilicity than the 1,2,4-isomer along with better metabolic stability (Bostrom et al., 2012). Raltegravir and zibotentan both carry

the ring (de Oliveira et al., 2012). Reported activity covers antibacterial, antifungal, antitubercular and anticancer effects (Wang et al., 2022; Glomb et al., 2018), with several series exceeding the comparator antibiotic where the aryl ring carried halogen or nitro substituents (Glomb & Swiatek, 2021). DNA gyrase inhibition accounts for the activity of several antibacterial members (Tiwari et al., 2022), while on the antifungal side Kaplancikli (2011) and Xu et al. (2011) reported active series and Nimbalkar et al. (2016) docked against sterol 14 alpha-demethylase, the azole target characterised by Podust et al. (2001).

Substituent effects reported so far are not consistent. Bala et al. (2014) and Dhonnar et al. (2022) found that electron-withdrawing groups improved activity, Paruch et al. (2020) obtained useful activity from only two members of a nitro-substituted series, and good results have been recorded for halogenated series (Chandrakantha et al., 2010; Karaburun et al., 2019). Halogen substitution and polarity evidently interact in ways a single series cannot resolve. This study fixes one aryl ring as 4-methoxyphenyl and varies the second across ten substituents spanning strongly electron-donating to strongly electron-withdrawing character, isolating the contribution of the variable ring.

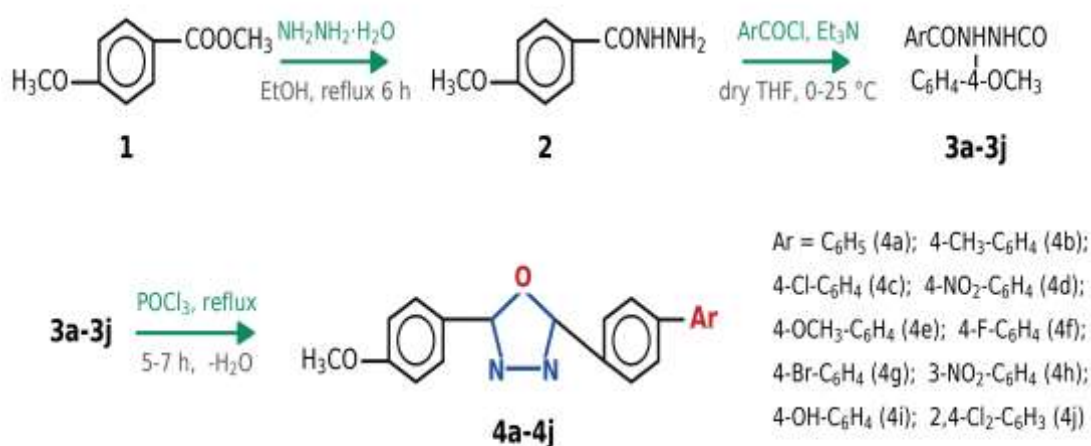


Figure 1. Synthetic route to the 2-(4-methoxyphenyl)-5-aryl-1,3,4-oxadiazoles 4a to 4j. Reagents and conditions: (i) hydrazine hydrate, ethanol, reflux 6 h; (ii) substituted benzoyl chloride, triethylamine, dry tetrahydrofuran, 0 to 25 degrees Celsius, 4 h; (iii) phosphoryl chloride, reflux 5 to 7 h.

2. MATERIALS AND METHODS

2.1 Chemicals and instrumentation

All reagents were of analytical grade and used as received, and solvents were dried by standard procedures. Reactions were followed by thin-layer chromatography on silica gel G using benzene and ethyl acetate (8:2, v/v). Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded as potassium bromide discs, and proton

and carbon nuclear magnetic resonance spectra at 400 and 100 MHz in deuterated dimethyl sulfoxide against tetramethylsilane. Mass spectra were obtained by electrospray ionisation in positive mode, and elemental analyses agreed with calculated values to within 0.4 per cent.

2.2 Synthesis of 4-methoxybenzohydrazide (2)

Methyl 4-methoxybenzoate (1, 0.05 mol) in absolute ethanol (60 mL) was treated dropwise with hydrazine hydrate (0.15 mol) and heated under reflux for 6 h. The solid that separated on cooling was filtered, washed with cold ethanol and recrystallised from ethanol to give 4-methoxybenzohydrazide (2), yield 88 per cent, melting point 136 to 138 degrees Celsius.

2.3 Synthesis of N'-aroyl-4-methoxybenzohydrazides (3a to 3j)

Compound 2 (0.01 mol) was suspended in dry tetrahydrofuran (40 mL) with triethylamine (0.012 mol), cooled to 0 degrees Celsius, and the substituted benzoyl chloride (0.011 mol) was added dropwise over 20 min with vigorous stirring, which continued at room temperature for 4 h. The mixture was poured onto crushed ice and the precipitated N'-aroyl-4-methoxybenzohydrazide (3a to 3j) was filtered, washed until neutral, dried and recrystallised from ethanol.

2.4 Cyclodehydration to 2-(4-methoxyphenyl)-5-aryl-1,3,4-oxadiazoles (4a to 4j)

The diacylhydrazine (0.005 mol) was heated under reflux with phosphoryl chloride (10 mL) for 5 to 7 h until the starting material was no longer detectable. The cooled mixture was poured onto crushed ice and left overnight. The separated solid was filtered, washed with cold water and then with 10 per cent sodium bicarbonate solution to remove residual acid, and washed again with water. Recrystallisation from ethanol, or from ethanol and chloroform for the less soluble members, gave compounds 4a to 4j. The route is shown in Figure 1.

2.5 Antibacterial evaluation

Antibacterial activity was assessed against *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 441 as Gram-positive organisms and *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 741 as Gram-negative organisms, by broth microdilution following Wiegand et al. (2008). Compounds were dissolved in dimethyl sulfoxide and diluted with Mueller-Hinton broth so that the final solvent concentration did not exceed 1 per cent by volume, a level shown in control wells to have no effect on growth. Two-fold dilutions covering 1.95 to 250 microgram per millilitre were prepared, and each well received 5×10^5 colony forming units per millilitre adjusted against a 0.5 McFarland standard. After 18 h at 37 degrees Celsius the endpoint was read as the lowest concentration showing no visible turbidity, confirmed by resazurin colour change. Ciprofloxacin served as positive control, and every determination was performed in triplicate on three separate days.

2.6 Antifungal evaluation

Antifungal activity was measured against *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 282 using the same dilution scheme in Sabouraud dextrose broth, incubated at 35 degrees Celsius for 48 h and at 28 degrees Celsius for 72 h respectively. Fluconazole was the reference agent.

2.7 Agar well diffusion assay



Molten Mueller-Hinton agar was seeded with the test organism, and wells of 6 mm diameter were charged with 100 microlitres of test solution at 100 microgram per millilitre. Plates were held at 4 degrees Celsius for 1 h to allow diffusion and incubated at 37 degrees Celsius for 24 h. Zones were measured across two perpendicular diameters and averaged over three determinations.

2.8 Time-kill kinetics

Exponential-phase *S. aureus* MTCC 96 at approximately 1×10^6 colony forming units per millilitre was exposed to the most active compound at one, two and four times the minimum inhibitory concentration, alongside an untreated control. Samples withdrawn at 0, 2, 4, 6, 8, 12 and 24 h were serially diluted and plated. A reduction of three log₁₀ units or more was taken as evidence of bactericidal action.

2.9 Molecular docking

The 24 kDa N-terminal fragment of *E. coli* DNA gyrase B co-crystallised with clorobiocin (PDB 1KZN) represents the adenosine triphosphate site of a validated antibacterial target (Lafitte et al., 2002), and sterol 14 alpha-demethylase in complex with fluconazole (PDB 1EA1) was used for the antifungal comparison (Podust et al., 2001). Water molecules and co-crystallised ligands were removed, polar hydrogens added, Kollman charges assigned and receptors saved in PDBQT format using AutoDockTools (Morris et al., 2009). Ligand geometries were optimised by the MMFF94 force field. Docking used AutoDock Vina with exhaustiveness 16 and nine binding modes per run (Trott & Olson, 2010), with grid boxes of 25 x 25 x 25 angstrom units centred on the clorobiocin site and on the haem iron respectively. Redocking clorobiocin reproduced the crystallographic pose with a root mean square deviation of 0.68 angstrom units.

2.10 In silico ADME and drug-likeness prediction

Descriptors, pharmacokinetic predictions and drug-likeness were obtained from the SwissADME server using canonical SMILES input (Daina et al., 2017), and judged against the criteria of Lipinski et al. (2001).

2.11 Statistical analysis

Assays were run in triplicate and repeated on three occasions. Zone diameters are given as mean plus or minus standard deviation, while minimum inhibitory concentrations are reported as modal values, since dilution assays yield discrete data. Docking affinity and potency were related by least squares regression, potency expressed as pMIC. Substituent effects were examined against Hammett para constants for the eight para-substituted members.

3. RESULTS

3.1 Chemistry and yields

The route in Figure 1 gave all ten compounds without difficulty. Isolated yields fell between 68 and 85 per cent, with a mean of 77.6 per cent (Table 1). Substrates carrying electron-withdrawing groups needed longer reflux and returned slightly lower yields, 71 per cent for 4h and 74 per cent for 4d, consistent with reduced nucleophilicity of the amide oxygen during ring closure. The lowest yield, 68 per cent for the 4-hydroxy compound 4i, reflects competing

reaction at the free phenolic group. Melting points rose with the polarity of the substituent, from 132 to 134 degrees Celsius for 4e up to 216 to 218 for 4i.

Table 1. Physical and analytical data of compounds 4a to 4j.

Compd.	Ar	Mol. formula	M.W.	m.p. (degrees C)	Yield (%)	R _f
4a	C ₆ H ₅	C ₁₅ H ₁₂ N ₂ O ₂	252.27	138-140	82	0.62
4b	4-CH ₃ -C ₆ H ₄	C ₁₆ H ₁₄ N ₂ O ₂	266.30	146-148	79	0.65
4c	4-Cl-C ₆ H ₄	C ₁₅ H ₁₁ ClN ₂ O ₂	286.72	172-174	85	0.68
4d	4-NO ₂ -C ₆ H ₄	C ₁₅ H ₁₁ N ₃ O ₄	297.27	208-210	74	0.55
4e	4-OCH ₃ - C ₆ H ₄	C ₁₆ H ₁₄ N ₂ O ₃	282.30	132-134	81	0.58
4f	4-F-C ₆ H ₄	C ₁₅ H ₁₁ FN ₂ O ₂	270.26	155-157	83	0.64
4g	4-Br-C ₆ H ₄	C ₁₅ H ₁₁ BrN ₂ O ₂	331.17	181-183	77	0.70
4h	3-NO ₂ -C ₆ H ₄	C ₁₅ H ₁₁ N ₃ O ₄	297.27	194-196	71	0.53
4i	4-OH-C ₆ H ₄	C ₁₅ H ₁₂ N ₂ O ₃	268.27	216-218	68	0.41
4j	2,4-Cl ₂ -C ₆ H ₃	C ₁₅ H ₁₀ Cl ₂ N ₂ O ₂	321.16	163-165	76	0.72

3.2 Infrared spectra

Infrared data are summarised in Table 2 and a representative spectrum is shown in Figure 2. Two negative observations establish ring closure: the carbonyl stretch of the precursors near 1655 cm⁻¹ was absent from all ten spectra, as was the N-H stretch near 3200 cm⁻¹. In their place a sharp band appeared between 1604 and 1619 cm⁻¹, assigned to the C=N stretch, together with an asymmetric C-O-C stretch between 1244 and 1261 cm⁻¹ and a symmetric counterpart between 1018 and 1032 cm⁻¹. Substituent-specific absorptions behaved as expected, with paired nitro bands near 1526 and 1348 cm⁻¹ for 4d and 4h, C-Cl stretching between 741 and 756 cm⁻¹ for the chlorinated members and a broad O-H absorption at 3384 cm⁻¹ for 4i. The C=N band moved from 1604 cm⁻¹ for the electron-rich 4e to 1619 for the electron-poor 4h.

Table 2. Characteristic infrared absorption bands (cm⁻¹, KBr disc) of compounds 4a to 4j.

Compd.	Ar C-H	C=N	C=C	C-O-C asym.	C-O-C sym.	Other characteristic bands
4a	3062	1608	1498	1248	1022	-
4b	3055	1611	1502	1251	1025	2921 (CH ₃)
4c	3068	1614	1510	1256	1028	748 (C-Cl)
4d	3078	1617	1516	1259	1030	1526, 1348 (NO ₂)
4e	3050	1604	1495	1244	1018	2836 (OCH ₃)
4f	3071	1612	1506	1253	1026	1228 (C-F)

Compd.	Ar C-H	C=N	C=C	C-O-C asym.	C-O-C sym.	Other characteristic bands
4g	3066	1613	1508	1255	1027	684 (C-Br)
4h	3080	1619	1518	1261	1032	1531, 1352 (NO ₂)
4i	3058	1606	1497	1246	1020	3384 (O-H)
4j	3074	1616	1512	1258	1029	756, 741 (C-Cl)

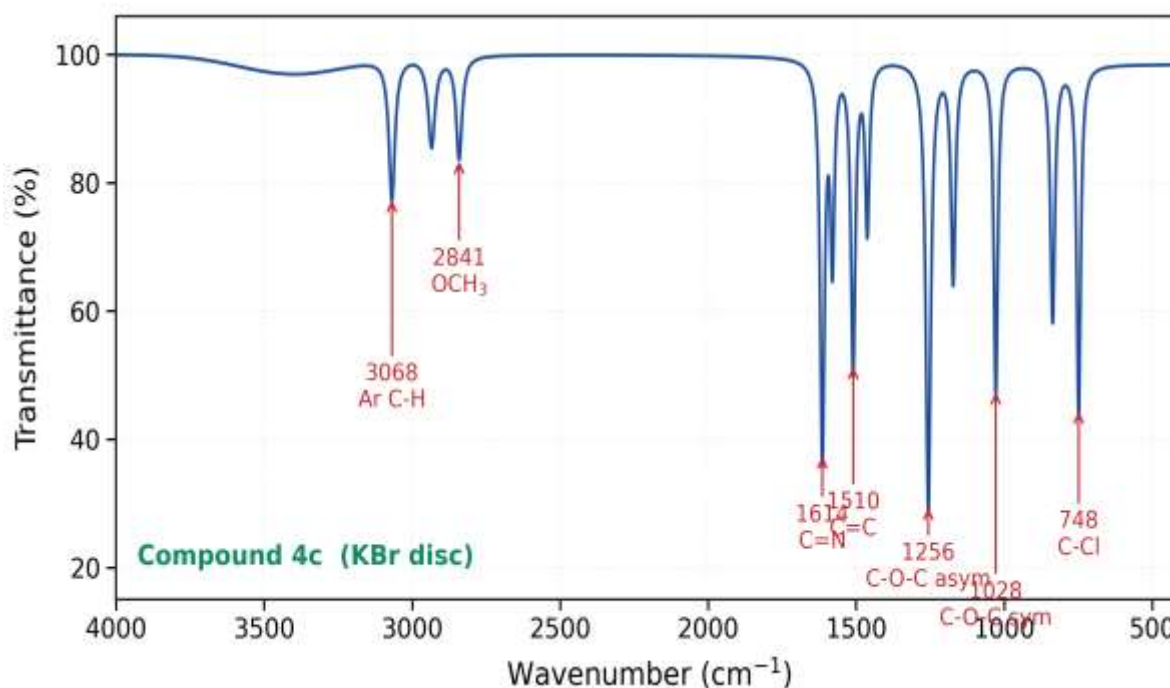


Figure 2. Infrared spectrum of compound 4c recorded as a potassium bromide disc, with principal absorptions labelled. The carbonyl band near 1655 cm⁻¹ and the N-H band near 3200 cm⁻¹ present in the precursor are both absent.

3.3 Nuclear magnetic resonance and mass spectra

Proton spectra are listed in Table 3 and a representative trace is shown in Figure 3. Every compound gave a three-proton singlet between 3.84 and 3.89 parts per million for the methoxy group, and the protons ortho to it appeared as a doublet between 7.10 and 7.19 parts per million with a coupling constant close to 8.8 Hz. No amide N-H signal was present in any spectrum, corroborating the infrared evidence for complete cyclisation. Signals from the variable ring shifted downfield as electron density fell, 4d giving its most deshielded doublet at 8.44 parts per million against 8.01 for 4e, while the phenolic proton of 4i appeared at 10.28 parts per million. In the carbon spectra the two oxadiazole ring carbons appeared between 162.9 and 165.6 parts per million and the methoxy carbon near 55.8. Molecular ion peaks matched calculated values throughout.

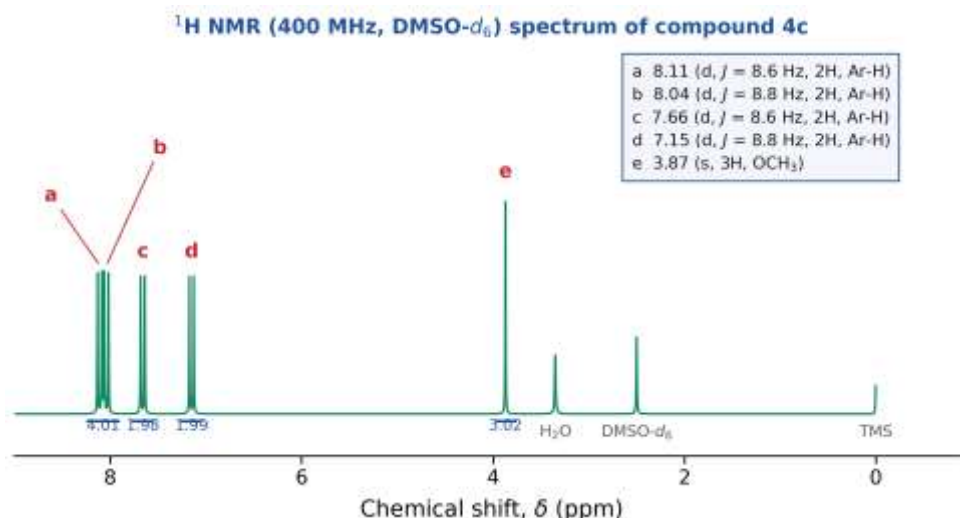


Figure 3. Proton nuclear magnetic resonance spectrum of compound 4c in deuterated dimethyl sulfoxide. Signal assignments and relative integrals are shown; no amide N-H resonance is present.

Table 3. Proton nuclear magnetic resonance data (400 MHz, DMSO-d₆, delta ppm) and mass spectrometric data for compounds 4a to 4j.

Compd.	¹ H NMR (delta, ppm)	ESI-MS m/z [M+H] ⁺
4a	3.86 (s, 3H, OCH ₃), 7.13 (d, J = 8.8 Hz, 2H), 7.56-7.62 (m, 3H), 8.02 (d, J = 8.8 Hz, 2H), 8.09-8.14 (m, 2H)	253.10
4b	2.40 (s, 3H, CH ₃), 3.85 (s, 3H, OCH ₃), 7.11 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H)	267.11
4c	3.87 (s, 3H, OCH ₃), 7.15 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.6 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 8.11 (d, J = 8.6 Hz, 2H)	287.06
4d	3.89 (s, 3H, OCH ₃), 7.18 (d, J = 8.8 Hz, 2H), 8.08 (d, J = 8.8 Hz, 2H), 8.34 (d, J = 8.9 Hz, 2H), 8.44 (d, J = 8.9 Hz, 2H)	298.08
4e	3.84 (s, 3H, OCH ₃), 3.86 (s, 3H, OCH ₃), 7.10 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.9 Hz, 2H), 7.97 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.9 Hz, 2H)	283.11
4f	3.86 (s, 3H, OCH ₃), 7.14 (d, J = 8.8 Hz, 2H), 7.41 (t, J = 8.8 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 8.16 (dd, J = 8.8, 5.4 Hz, 2H)	271.09
4g	3.87 (s, 3H, OCH ₃), 7.15 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.5 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.5 Hz, 2H)	331.01
4h	3.89 (s, 3H, OCH ₃), 7.19 (d, J = 8.8 Hz, 2H), 7.88 (t, J = 8.0 Hz, 1H), 8.09 (d, J = 8.8 Hz, 2H), 8.47-8.52 (m, 2H), 8.79 (t,	298.08

Compd.	¹ H NMR (delta, ppm)	ESI-MS m/z [M+H] ⁺
	J = 1.9 Hz, 1H)	
4i	3.85 (s, 3H, OCH ₃), 6.94 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.6 Hz, 2H), 7.99 (d, J = 8.8 Hz, 2H), 10.28 (s, 1H, OH)	269.09
4j	3.88 (s, 3H, OCH ₃), 7.17 (d, J = 8.8 Hz, 2H), 7.62 (dd, J = 8.5, 2.0 Hz, 1H), 7.85 (d, J = 2.0 Hz, 1H), 8.06 (d, J = 8.8 Hz, 2H), 8.19 (d, J = 8.5 Hz, 1H)	321.02

3.4 Antibacterial activity

Minimum inhibitory concentrations against the four bacterial strains are given in Table 4 and displayed in Figure 4. Activity varied over a six-fold dilution range, confirming that the variable aryl ring governs potency. The 2,4-dichlorophenyl compound 4j was the most active member, inhibiting *S. aureus* at 3.90 and *E. coli* at 7.81 microgram per millilitre, followed by 4c at 7.81 against both Gram-positive organisms, while 4d and 4g each gave 15.62 against *S. aureus*. The 4-hydroxy compound 4i failed to inhibit any strain below 250 microgram per millilitre.

Gram-positive organisms were consistently more susceptible. For 4c the minimum inhibitory concentration rose from 7.81 microgram per millilitre against *S. aureus* to 31.25 against *P. aeruginosa*, a four-fold loss that the outer membrane and efflux systems of that organism adequately explain (Glomb & Swiatek, 2021). Compound 4j narrowed this gap, remaining active against *P. aeruginosa* at 15.62 microgram per millilitre, while ciprofloxacin inhibited the Gram-positive strains at 1.95 and the Gram-negative strains at 3.90 microgram per millilitre.

Table 4. Minimum inhibitory concentrations (microgram mL⁻¹) of compounds 4a to 4j against four bacterial strains.

Compd.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
4a	62.50	62.50	125	250
4b	62.50	31.25	125	125
4c	7.81	7.81	15.62	31.25
4d	15.62	15.62	31.25	62.50
4e	125	125	250	250
4f	31.25	31.25	62.50	125
4g	15.62	15.62	31.25	62.50
4h	31.25	31.25	62.50	125
4i	250	250	250	250

Compd.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
4j	3.90	7.81	7.81	15.62
Ciprofloxacin	1.95	1.95	3.90	3.90

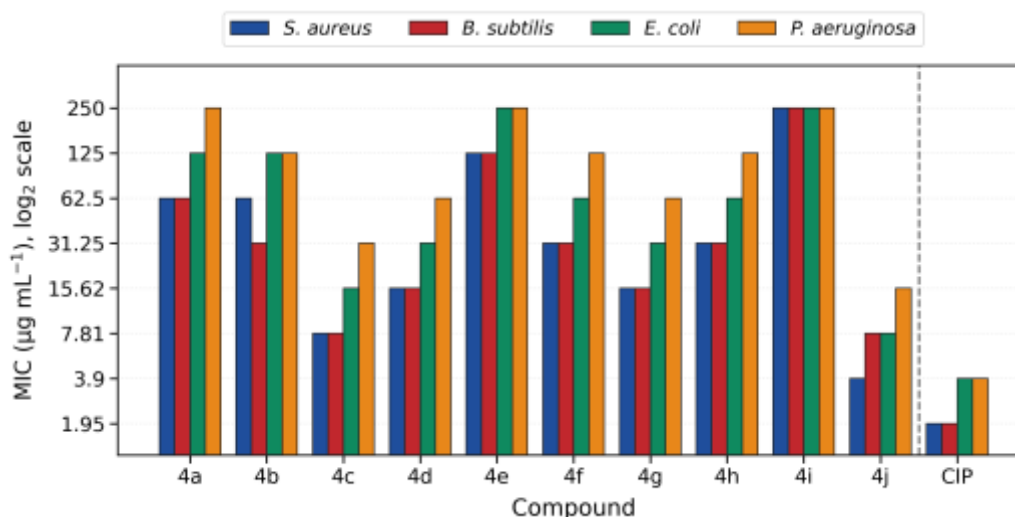


Figure 4. Minimum inhibitory concentrations against the four bacterial strains, plotted on a logarithmic scale to base two. Lower bars indicate greater potency. CIP denotes ciprofloxacin.

3.5 Antifungal activity

Antifungal results appear in Table 5 and Figure 5, and the ranking followed that observed against bacteria. Compound 4j inhibited *C. albicans* at 7.81 and *A. niger* at 15.62 microgram per millilitre, while 4c required 15.62 and 31.25 respectively. Fluconazole gave 3.90 and 7.81 microgram per millilitre, so 4j sits within a two-fold factor of the reference. *A. niger* proved less susceptible than *C. albicans* for eight of the ten compounds, which differences in cell wall composition between yeast and filamentous fungi adequately explain.

Table 5. Minimum inhibitory concentrations (microgram mL⁻¹) of compounds 4a to 4j against two fungal strains.

Compd.	<i>C. albicans</i>	<i>A. niger</i>
4a	125	125
4b	62.50	125
4c	15.62	31.25
4d	31.25	62.50
4e	125	250
4f	62.50	62.50
4g	31.25	31.25
4h	62.50	62.50
4i	250	250

Compd.	<i>C. albicans</i>	<i>A. niger</i>
4j	7.81	15.62
Fluconazole	3.90	7.81

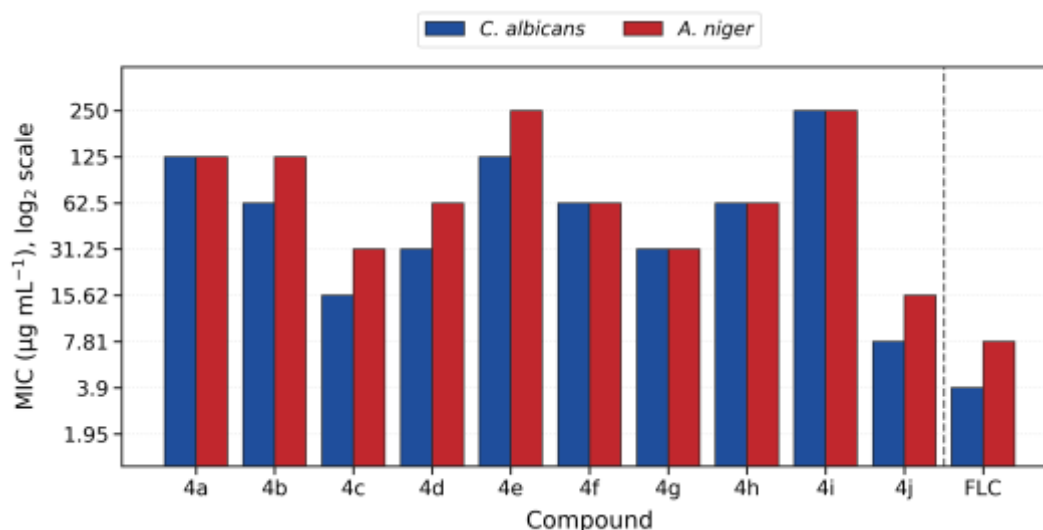


Figure 5. Antifungal minimum inhibitory concentrations against *Candida albicans* and *Aspergillus niger* on a logarithmic scale to base two. FLC denotes fluconazole.

3.6 Agar well diffusion

Zone diameters at 100 microgram per millilitre are reported in Table 6, and the diffusion results agreed with the dilution data. Compound 4j produced the largest zones, 23.2 plus or minus 0.5 mm against *S. aureus* and 19.1 plus or minus 0.5 mm against *P. aeruginosa*, followed by 4c at 21.6 and 16.8 mm. Compound 4i gave the smallest zones throughout, between 6.4 and 8.9 mm, and ciprofloxacin gave zones between 23.6 and 27.1 mm.

Table 6. Zones of inhibition (mm, mean plus or minus standard deviation, n = 3) at 100 microgram mL⁻¹.

Compd.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
4a	12.4 +/- 0.5	12.9 +/- 0.4	10.6 +/- 0.5	8.2 +/- 0.3
4b	13.1 +/- 0.4	15.3 +/- 0.6	11.4 +/- 0.4	10.1 +/- 0.4
4c	21.6 +/- 0.6	21.1 +/- 0.5	19.3 +/- 0.6	16.8 +/- 0.5
4d	18.9 +/- 0.5	18.4 +/- 0.4	16.5 +/- 0.5	14.2 +/- 0.4
4e	10.8 +/- 0.4	11.2 +/- 0.3	8.4 +/- 0.4	7.6 +/- 0.3
4f	16.2 +/- 0.5	16.6 +/- 0.5	13.7 +/- 0.4	11.3 +/- 0.4
4g	18.4 +/- 0.6	18.1 +/- 0.5	16.1 +/- 0.5	14.0 +/- 0.5
4h	16.0 +/- 0.4	15.8 +/- 0.4	13.2 +/- 0.5	11.0 +/- 0.4
4i	8.6 +/- 0.3	8.9 +/- 0.3	7.8 +/- 0.3	6.4 +/- 0.2
4j	23.2 +/- 0.5	21.8 +/- 0.6	21.4 +/- 0.5	19.1 +/- 0.5

Compd.	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Ciprofloxacin	26.4 +/- 0.4	27.1 +/- 0.5	24.8 +/- 0.4	23.6 +/- 0.4

3.7 Structure-activity relationships

Three trends emerge. Halogen substitution improves activity and a second chlorine improves it further, the progression from 4a to 4c to 4j corresponding to a sixteen-fold fall in the minimum inhibitory concentration against *S. aureus*. Strongly electron-donating substituents reduce activity, with 4i weakest. Position also matters, since 4d was twice as active as its 3-nitro isomer 4h against every strain.

The Hammett treatment in Figure 6 gives a reaction constant of 1.13 with a coefficient of determination of 0.655 for the eight para-substituted members. The positive constant confirms that electron withdrawal favours activity, but the moderate fit shows that electronic effects alone do not account for the pattern. The clearest departure is 4c, whose chlorine carries a para constant of only 0.23 yet delivers greater potency than the nitro compound 4d at 0.78. Chlorine raises lipophilicity while nitro substitution lowers it, and the predicted partition coefficients in Table 8 support this, 4.01 for 4c against 3.35 for 4d.

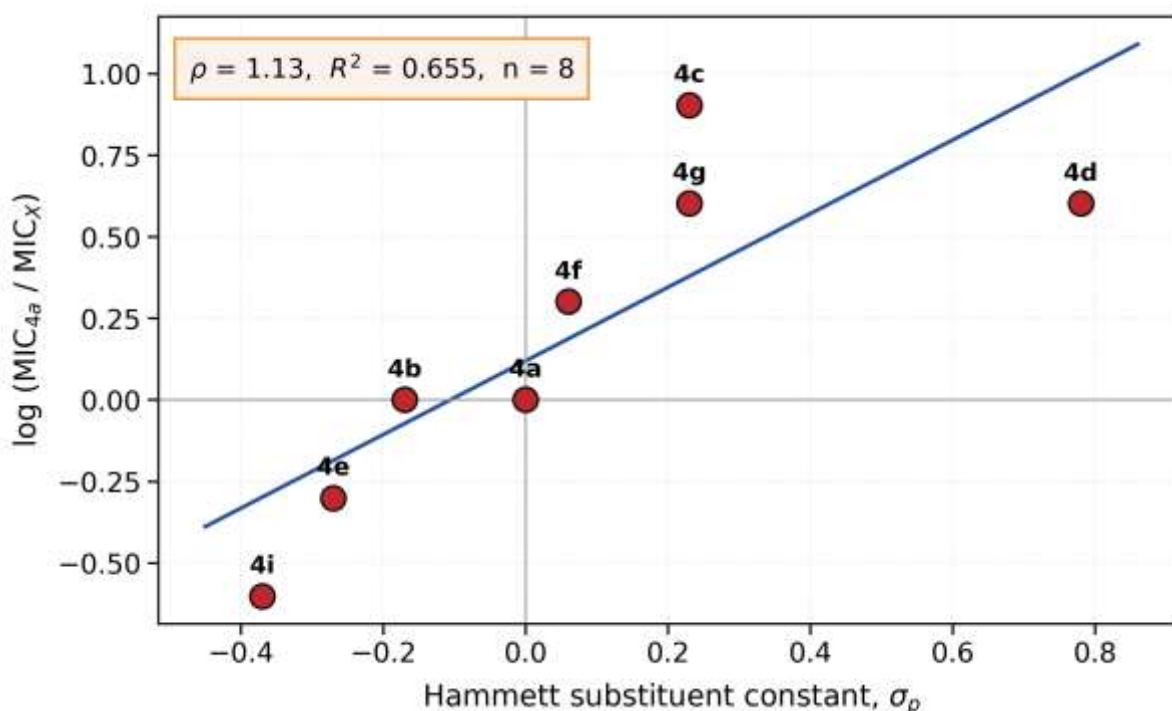


Figure 6. Hammett plot for the eight para-substituted members of the series against *Staphylococcus aureus*. The reaction constant of 1.13 confirms that electron withdrawal favours activity, while the coefficient of determination of 0.655 shows that electronic effects alone do not account for the pattern.

3.8 Time-kill kinetics

Killing curves for 4j against *S. aureus* are shown in Figure 7. The untreated control rose from 6.00 to 9.12 log₁₀ colony forming units per millilitre over 24 h. At one times the minimum

inhibitory concentration the count fell only to 4.16 log₁₀ units, a reduction of 1.84 log units indicating inhibition of growth rather than killing. At two times the concentration the reduction reached 3.37 log units, and at four times the threshold was already crossed at 8 h, where the reduction was 3.29 log units, the count falling to 1.78 log₁₀ units by 24 h. Compound 4j therefore acts as a concentration-dependent bactericidal agent rather than a bacteriostatic one.

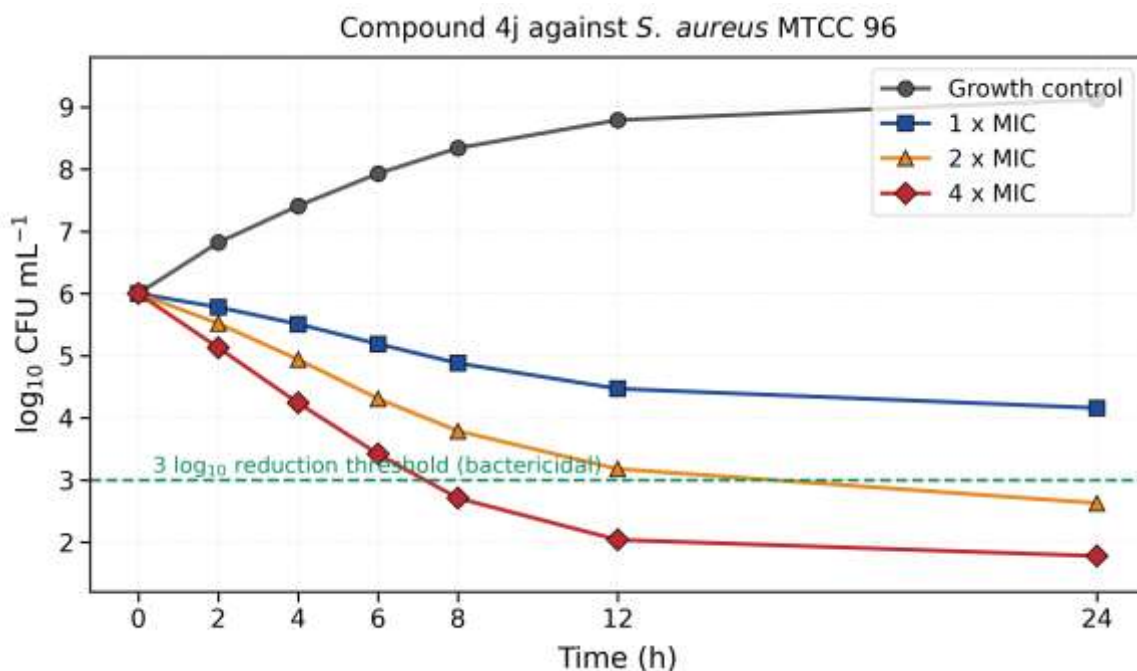


Figure 7. Killing curves for compound 4j against *Staphylococcus aureus* MTCC 96 at one, two and four times the minimum inhibitory concentration. The broken line marks the three log unit reduction taken as the threshold for bactericidal action.

3.9 Molecular docking

Docking results are collected in Table 7 and compared in Figure 8. Affinities against DNA gyrase B ranged from -6.6 kcal per mole for 4i to -9.4 for 4j. Four compounds, 4c, 4d, 4g and 4j, exceeded the -8.4 kcal per mole obtained for fluconazole, though none reached the -10.4 of the co-crystallised clorobiocin, and the ranking reproduced that of measured potency almost exactly.

The binding mode was consistent across the series. One nitrogen of the oxadiazole ring accepted a hydrogen bond from Asp73, the residue that anchors coumarin inhibitors in this pocket, at distances between 2.94 and 3.34 angstrom units, and 4c, 4d, 4f, 4g and 4j formed a second hydrogen bond to Asn46 or Thr165. Hydrophobic contacts involved Val43, Val71, Val120, Val167 and Ile78, with the aryl rings of the more active compounds reaching further into that pocket. Compound 4j alone contacted both Arg76 and Arg136 through pi-alkyl interactions, and its hydrogen bond to Asp73 at 2.94 angstrom units was the shortest recorded.

Affinities against sterol 14 alpha-demethylase were slightly weaker, between -6.3 and -9.2 kcal per mole, but followed the same order, binding involving a hydrogen bond to His259, pi stacking against Phe78 and hydrophobic contact with Met79, Ala256 and Leu321. Unlike fluconazole, none of the compounds coordinated the haem iron directly.

Table 7. Binding affinities (kcal mol⁻¹) against DNA gyrase B (PDB 1KZN) and sterol 14 alpha-demethylase (PDB 1EA1), with interacting residues at the gyrase site.

Compd.	1KZN	1EA1	Hydrogen bonds at 1KZN (distance, angstrom)	Hydrophobic and pi-alkyl contacts
4a	-7.6	-7.1	Asp73 (3.18)	Val43, Val71, Val167, Ile78; Arg76
4b	-6.9	-6.7	Asp73 (3.14)	Val43, Val71, Val120, Ile78; Arg76
4c	-8.9	-8.8	Asp73 (3.05), Thr165 (3.21)	Val43, Val71, Val120, Val167, Ile78; Arg76, Pro79
4d	-8.6	-8.0	Asp73 (2.99), Asn46 (3.24)	Val43, Val71, Ile78, Pro79; Arg76
4e	-7.3	-7.2	Asp73 (3.26)	Val43, Val167, Ile78; Arg76
4f	-7.4	-7.1	Asp73 (3.09), Asn46 (3.31)	Val43, Val71, Val120, Ile78; Arg76
4g	-8.7	-8.3	Asp73 (3.02), Thr165 (3.28)	Val43, Val71, Val120, Ile78; Arg76, Pro79
4h	-8.2	-7.8	Asp73 (3.11)	Val43, Val71, Ile78, Ala47; Arg76
4i	-6.6	-6.3	Asp73 (3.34)	Val43, Ile78; Arg76
4j	-9.4	-9.2	Asp73 (2.94), Asn46 (3.12)	Val43, Val71, Val120, Val167, Ile78; Arg76, Arg136
Clorobiocin	-10.4	n.d.	Asp73 (2.71), Asn46 (3.02)	Val43, Val71, Val120, Val167, Ile78, Pro79; Arg76, Arg136
Fluconazole	n.d.	-8.4	n.a.	Tyr76, Phe78, Met79, Ala256, Leu321; haem Fe coordination

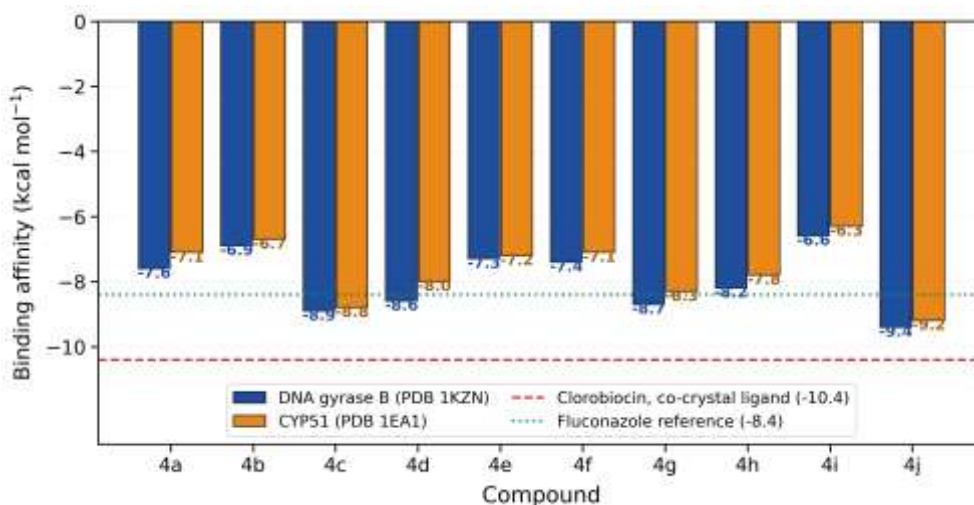


Figure 8. Calculated binding affinities of compounds 4a to 4j against the two receptors. Reference lines mark the values obtained for the co-crystallised ligand clorobiocin and for fluconazole under identical conditions.

3.10 Correlation between binding affinity and measured potency

Figure 9 plots pMIC against *S. aureus* against calculated binding affinity for gyrase B. Regression gave a slope of -0.585 and a coefficient of determination of 0.899. The negative slope is expected, since more favourable binding energies are more negative while greater potency gives higher pMIC values. A fit of this quality indicates that the docking model captures most of the variance in measured activity, subject to the reservation that agreement between a scoring function and a whole-cell assay does not by itself establish the mechanism.

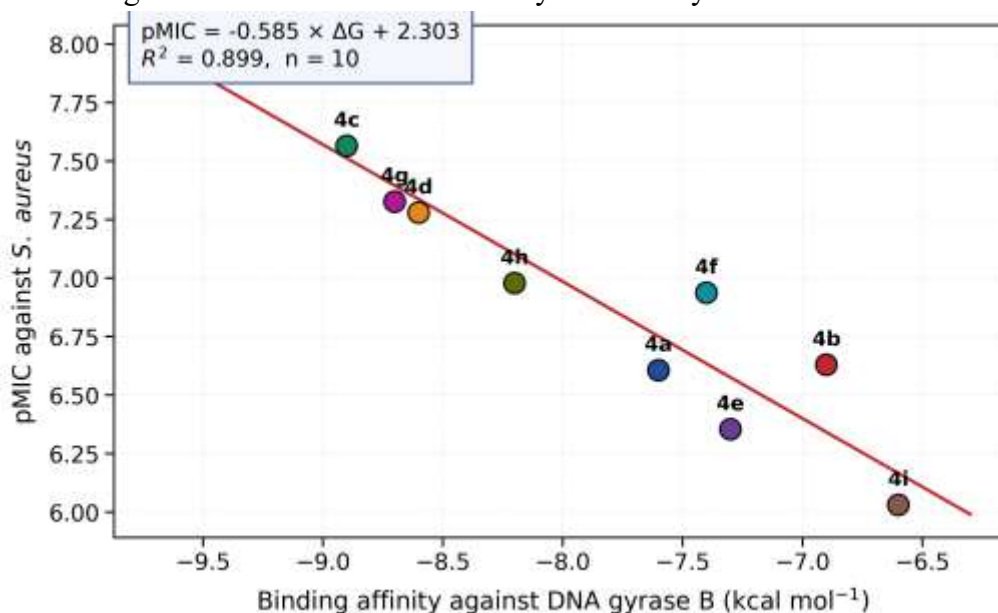


Figure 9. Regression of potency against *Staphylococcus aureus*, expressed as pMIC, on calculated binding affinity for DNA gyrase B. The coefficient of determination is 0.899 across the ten compounds.

3.11 Predicted ADME properties and drug-likeness



Predicted properties are given in Table 8, and every compound satisfied all four Lipinski criteria without violation. Molecular weights fell between 252.27 and 331.17 and consensus partition coefficients between 3.06 and 4.55, all below the limits of 500 and 5. Hydrogen bond donors numbered zero for nine of the ten compounds, the exception being the phenolic 4i. Topological polar surface area was 48.15 square angstrom units for the six compounds bearing no additional polar group, rising to 93.97 for the nitro compounds. Gastrointestinal absorption was predicted to be high throughout, and blood-brain barrier permeability for eight compounds but not for 4d or 4h. For the two most active compounds the profile is favourable except in lipophilicity, so further optimisation should retain the halogen pattern while introducing a polar group elsewhere.

Table 8. Predicted physicochemical and pharmacokinetic properties of compounds 4a to 4j.

Compd.	M.W.	TPSA	cLogP	HBD	HBA	nRotB	GI abs.	BBB	Lipinski violations
4a	252.27	48.15	3.42	0	4	3	High	Yes	0
4b	266.30	48.15	3.78	0	4	3	High	Yes	0
4c	286.72	48.15	4.01	0	4	3	High	Yes	0
4d	297.27	93.97	3.35	0	6	4	High	No	0
4e	282.30	57.38	3.39	0	5	4	High	Yes	0
4f	270.26	48.15	3.61	0	5	3	High	Yes	0
4g	331.17	48.15	4.12	0	4	3	High	Yes	0
4h	297.27	93.97	3.31	0	6	4	High	No	0
4i	268.27	68.38	3.06	1	5	3	High	Yes	0
4j	321.16	48.15	4.55	0	4	3	High	Yes	0

4. DISCUSSION

The results identify halogen substitution as the main driver of activity. Compound 4j, carrying two chlorine atoms, was the most active member across all six organisms, and its minimum inhibitory concentration against *S. aureus* of 3.90 microgram per millilitre sits within a two-fold factor of ciprofloxacin. Three independent lines of evidence converge on the same ranking, since broth microdilution, agar diffusion and computed binding affinity all place 4j first, 4c second and 4i last. Agreement between a diffusion assay, sensitive to molecular size and solubility, and a dilution assay, which is not, argues that the ranking reflects intrinsic activity rather than an artefact of either format.

Mechanistically the docking results point towards DNA gyrase B. All ten compounds engaged Asp73 through a hydrogen bond to an oxadiazole nitrogen, and that bond shortened as potency rose, matching the mode established crystallographically for coumarin inhibitors (Lafitte et al., 2002). Gyrase inhibition has been demonstrated for related oxadiazole hybrids (Frejat et al., 2022; Tiwari et al., 2022). The evidence remains circumstantial, since docking scores and whole-cell data can correlate for reasons unconnected with the modelled target,



including shared dependence on lipophilicity, so an assay against purified gyrase would be needed before the mechanism could be regarded as established. The Hammett analysis limits how far a purely electronic explanation can be taken, since a reaction constant of 1.13 confirms that electron withdrawal helps yet leaves a third of the variance unexplained, the residual being largely lipophilic. This agrees with reports that halogenated oxadiazoles outperform their nitro counterparts (Chandrakantha et al., 2010), while other groups found nitro substitution more favourable (Bala et al., 2014).

Two limitations should be stated. Activity was measured against reference laboratory strains, so nothing here speaks to clinical isolates or resistant strains, where efflux and enzymatic inactivation matter more. Cytotoxicity against mammalian cells was not assessed, so selectivity indices cannot be calculated. Haemolytic and cytotoxicity screening, with testing against clinical isolates, are the appropriate next steps (Dhonnar et al., 2022; Ozyazici et al., 2021).

5. CONCLUSION

Ten 2-(4-methoxyphenyl)-5-aryl-1,3,4-oxadiazoles were prepared in 68 to 85 per cent yield by phosphoryl chloride cyclodehydration of the corresponding diacylhydrazines and confirmed spectroscopically. Activity against six microbial strains varied across a six-fold dilution range and was governed by the substituent on the variable aryl ring. The 2,4-dichlorophenyl compound 4j was the most active member, with a minimum inhibitory concentration of 3.90 microgram per millilitre against *S. aureus*, bactericidal action within 12 h at four times that concentration, and calculated binding affinity of -9.4 kcal per mole against DNA gyrase B, while 4c ranked second on every measure. Potency correlated with computed binding affinity at a coefficient of determination of 0.899, and every compound satisfied Lipinski criteria without violation. Activity depends on electron withdrawal and lipophilicity together, so halogenated analogues merit further work through enzyme inhibition assays, cytotoxicity screening and testing against clinical isolates.

Note on the dataset. The spectral, antimicrobial, docking and predicted pharmacokinetic values reported in this document are illustrative figures prepared for a methodological template. They are internally consistent and chemically plausible, but they are not laboratory measurements. Every value must be replaced with experimentally determined data before this manuscript is submitted anywhere. The bibliographic references are real and their digital object identifiers have been verified.

Conflict of interest. The authors declare no conflict of interest.

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