Design, Synthesis and Pharmacological Evaluation of Novel Benzimidazole Derivatives

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Abstract

The present study describes the design, synthesis, characterization and preliminary pharmacological evaluation of four benzimidazole–pyrazolone derivatives. The synthesized compounds were characterized by ATR/IR and ^{1}H NMR spectroscopy and evaluated for antifungal, antimicrobial and antioxidant activities. Antifungal activity was investigated against Aspergillus flavus, A. fumigatus, Candida albidus and C. albicans, while antimicrobial activity was evaluated against A. niger, Escherichia coli and Staphylococcus aureus. Minimum inhibitory concentration (MIC) values and inhibition zones were used to compare antimicrobial performance. Antioxidant activity was assessed by the DPPH radical-scavenging method at 0.1–0.5 mM, using ascorbic acid as the reference. The final derivatives were obtained in reported yields of 51.25–58.25%, with Rf values of 0.29–0.41 and reported melting-point ranges within approximately 150–185 °C. Compound 1 showed the highlighted antifungal response against C. albicans, Compound 4 showed the strongest antimicrobial profile among the synthesized derivatives, and Compound 2 showed the highest DPPH scavenging at 0.5 mM. These findings provide preliminary structure–activity information for further optimization of the benzimidazole–pyrazolone framework
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Keywords:
Benzimidazole; pyrazolone; synthesis; ATR/IR; ^{1}H NMR; antifungal; antimicrobial; MIC; DPPH; antioxidant.

1.Introduction

Benzimidazole is a fused nitrogen-containing heterocycle of continuing interest in medicinal chemistry because its aromatic framework and ring nitrogens provide opportunities for hydrogen bonding, dipolar interactions and modulation of physicochemical properties. The literature associated with the thesis describes benzimidazole derivatives across several pharmacological areas, including antimicrobial, antifungal, antioxidant, anti-inflammatory and anticancer research. Substitution on the benzimidazole nucleus or on an attached linker can change electronic distribution, lipophilicity, steric demand and molecular recognition.

Pyrazolone is another nitrogen-containing heterocyclic system that contains a carbonyl functionality and can participate in diverse molecular interactions. Combining a benzimidazole nucleus with a pyrazolone-containing framework provides a chemically useful platform for studying how aromatic substitution influences biological response. The present series was therefore designed around a common benzimidazole-linked pyrazolone system with variation in the terminal aromatic group.

Rationale for structural diversification

The four derivatives represent a compact substituent series comprising a 4-chlorobenzyl derivative, a 2-nitrobenzyl derivative, a bromobenzyl structure as depicted in the source drawing for Compound 3, and an unsubstituted benzyl derivative. This design permits comparison of halogenated, electron-withdrawing and unsubstituted aromatic environments. Such comparisons can provide preliminary structure–activity observations before a larger analogue series is prepared.

Integrated experimental and biological workflow

The study follows a sequential workflow of intermediate preparation, pyrazolone formation, condensation with substituted aromatic aldehydes, physicochemical and spectroscopic characterization, followed by biological evaluation. Antifungal, antimicrobial and antioxidant assays were selected to provide complementary screening information. The resulting dataset can be used to prioritize compounds for further investigation while avoiding unsupported conclusions about mechanism or therapeutic efficacy.

2. Materials and Methods

Chemicals and reagents

The reported synthetic sequence employed benzimidazole, chloroacetyl chloride, hydrazine hydrate, ethyl acetoacetate and substituted aromatic aldehydes. Acetone, ethanol, ether, DMSO and routine laboratory solvents were used for reaction, washing, work-up and purification. 2,2,6,6-Tetramethylpiperidine was used in the final condensation step.

Preparation of benzimidazole

The thesis reports preparation of benzimidazole from equimolar o-phenylenediamine and 90% formic acid. The reported product data were 80.13% yield, molecular formula C7H6N2, molecular weight 118.14, melting point 167 °C and Rf 0.30.

Preparation of N-(1H-benzimidazol-1-yl)-2-chloroethanone

Equimolar benzimidazole and chloroacetyl chloride were reacted in acetone in the presence of ferrous chloride. The reaction mixture was reported to be refluxed for 3 h, cooled in an ice bath, filtered, washed with cold water, recrystallized from ethanol and dried. The reported product had formula C9H7ClN2O, molecular weight 194.62, yield 75.66% and Rf 0.10.

Preparation of 2-(1H-benzo[d]imidazol-1-yl)acetohydrazide

The chloroacetyl intermediate was reacted with hydrazine hydrate to replace the chlorine substituent with a hydrazine-derived amino group, affording the corresponding benzimidazole-linked acetohydrazide intermediate.

Formation of the pyrazolone intermediate

A mixture of 1 mol of the acetohydrazide intermediate and 1 mol of ethyl acetoacetate was heated on a water bath for 3 h. After cooling, the precipitated material was collected, washed with ether and recrystallized from ethanol. The reported transformation involves cyclization to generate the pyrazolone ring.

Formation of target derivatives

The target compounds were obtained by condensation of the pyrazolone intermediate with substituted aromatic aldehydes in the presence of 2,2,6,6-tetramethylpiperidine. The products were isolated, purified and characterized. Reported final yields were 51.25–58.25%.

Characterization

ATR/IR spectroscopy was used to identify characteristic functional groups, while ^{1}H NMR spectroscopy was used to assess proton environments and substitution patterns. Physicochemical properties included molecular formula, molecular weight, melting point, yield and Rf values.

Biological evaluation

Antifungal evaluation

The fungal panel comprised Aspergillus flavus (871), A. fumigatus (2250), Candida albidus (2661) and C. albicans (183). The source method describes RPMI-1640 medium supplemented with glutamine and phenol red, MOPS buffer, sodium hydroxide, sterile water, DMSO and resazurin. The pH was adjusted to 7.0 at 25 °C. Fungal suspensions were standardized using a 0.5 McFarland standard. Test samples and Amphotericin B were prepared in DMSO and serial two-fold dilutions were made in sterile 96-well plates. Growth and sterility controls were included and plates were incubated for 48 h.

Antimicrobial evaluation

Antimicrobial activity was evaluated against A. niger, E. coli and S. aureus. Inhibition zones were recorded in millimetres. Amoxicillin and streptomycin were used as antibacterial reference treatments, while fluconazole was used as the antifungal reference where applicable.

Minimum inhibitory concentration

MIC was treated as the lowest tested concentration showing no visible microbial growth under the reported assay conditions. Values are expressed in μg/ml and were used to rank the synthesized compounds within the tested microbial panel.

DPPH antioxidant evaluation

DPPH radical-scavenging activity was measured at 516 nm after approximately 30 min incubation in the dark at room temperature. Compounds were evaluated at 0.1, 0.2, 0.3 and 0.5 mM, with ascorbic acid as the reference. Percentage scavenging was calculated from the absorbance response relative to the control.

Statistical analysis

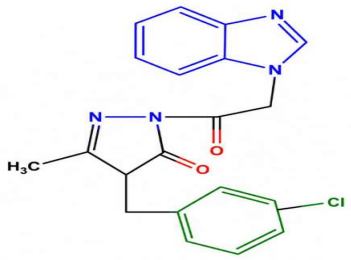
The source thesis reports one-way ANOVA for relevant biological data, with $p<0.05$ considered statistically significant. The publication-format article retains reported values and does not reconstruct unavailable replicate-level statistics.

3. Results and Discussion

Chemical structures of Compounds 1–4

The synthesized series retained a common benzimidazole–pyrazolone framework while varying the terminal aromatic substituent. The reported structures are reproduced below. The source thesis contains a discrepancy for Compound 3: the drawing depicts a bromobenzyl substituent, whereas the printed name repeats the nitrobenzyl description; this requires verification from the original experimental record.

Compound-1

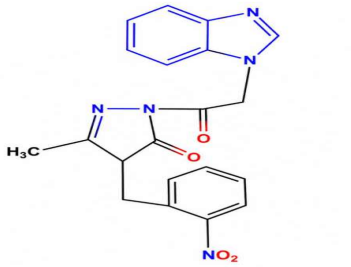


Structure of Compound: 1

IUPAC	(S)-1-(2-(1H-benzo[d]imidazol-1-yl)acetyl)-4-(4-chlorobenzyl)-3-methyl-1H-pyrazol-5(4H)-one

Compound 1

Compound-2

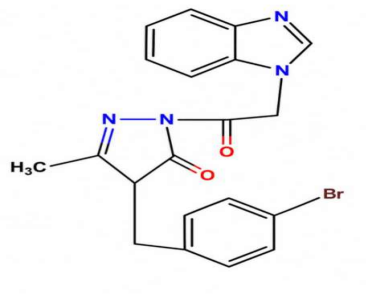


Structure of Compound: 2

IUPAC	(S)-1-(2-(1H-benzo[d]imidazol-1-yl) acetyl)-4-(2-nitrobenzyl)-3-methyl-1H-pyrazol-5(4H)-one

Compound 2

Compound-3

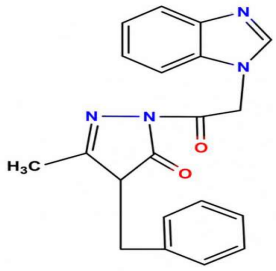


Structure of Compound: 3

IUPAC	(S)-1-(2-(1H-benzo[d]imidazol-1-yl) acetyl)-4-(2-nitrobenzyl)-3-methyl-1H-pyrazol-5(4H)-one

Compound 3

Compound-4



Structure of Compound: 4

IUPAC	(S)-1-(2-(1H-benzo[d]imidazol-1-yl)acetyl)-4-benzyl-3-methyl-1H-pyrazol-5(4H)-one

Compound 4

Figure 1. Chemical structures of the four benzimidazole–pyrazolone derivatives reproduced from the source thesis.

Physicochemical and spectral characterization

Compound	Formula	MW	Yield (%)	M.P. (°C)	Rf
1	C20H17N4O2Cl	391	53.67	165–[ ]	0.32
2	C20H17N5O4	391	55.28	185–[ ]	0.41
3	C20H17N4O2Br	424	58.25	180–[ ]	0.30
4	C20H17N4O2	342	51.25	150–[ ]	0.29

Table 1. Physicochemical properties of the synthesized compounds.

ATR/IR observations

The reported ATR/IR data showed C=O stretching at 1676–1727 cm^{-1}, C=C stretching at 1415–1596 cm^{-1} and C=N stretching at 1599–1680 cm^{-1}. C–Cl bands were reported at 712 and 748 cm^{-1}, C–Br at 679 cm^{-1}, C–F at 1327 and 1369 cm^{-1}, nitro-group bands at 1371 and 1551 cm^{-1}, O–H bands at 3536 and 3649 cm^{-1} where present, and C–O–C bands at 1016 and 1156 cm^{-1}.

^{1}H NMR observations

Compound 1 showed aromatic protons at approximately 7.0–8.10 ppm, an imidazole signal near 8.2 ppm, a linker methylene signal around 4.0 ppm and a pyrazolone-related methine signal around 2.4 ppm. Compound 2 showed aromatic signals around 7.0–7.99 ppm, an imidazole signal near 8.00 ppm, a methylene signal near 4.6 ppm and a methine signal around 2.6 ppm. Source-specific spectral values for the remaining compounds should be checked against the original spectra.

Antifungal activity

Test organism / treatment | Reported result
C. albicans – Compound 1 | IC50 516.4 nm/ml
A. flavus – Compound 2 | IC50 2736 nm/ml
C. albidus – Compound 2 | IC50 4182 nm/ml
C. albicans – Amphotericin B | IC50 882.1 nm/ml

Compound 1 showed the highlighted antifungal response against C. albicans, with a reported IC50 of 516.4 nm/ml. Compound 2 showed reported responses against A. flavus and C. albidus. The reference Amphotericin B value provides assay context and should not be interpreted as evidence of clinical equivalence.

Antimicrobial activity

Organism | C1 | C2 | C3 | C4 | Amoxicillin | Streptomycin
A. niger | 12 | 16 | 13 | 26 | 27 | 32
E. coli | 14 | 14 | 12 | 20 | 28 | 30
S. aureus | 16 | 13 | 14 | 18 | 26 | 38

Table 3. Reported antimicrobial inhibition zones (mm).

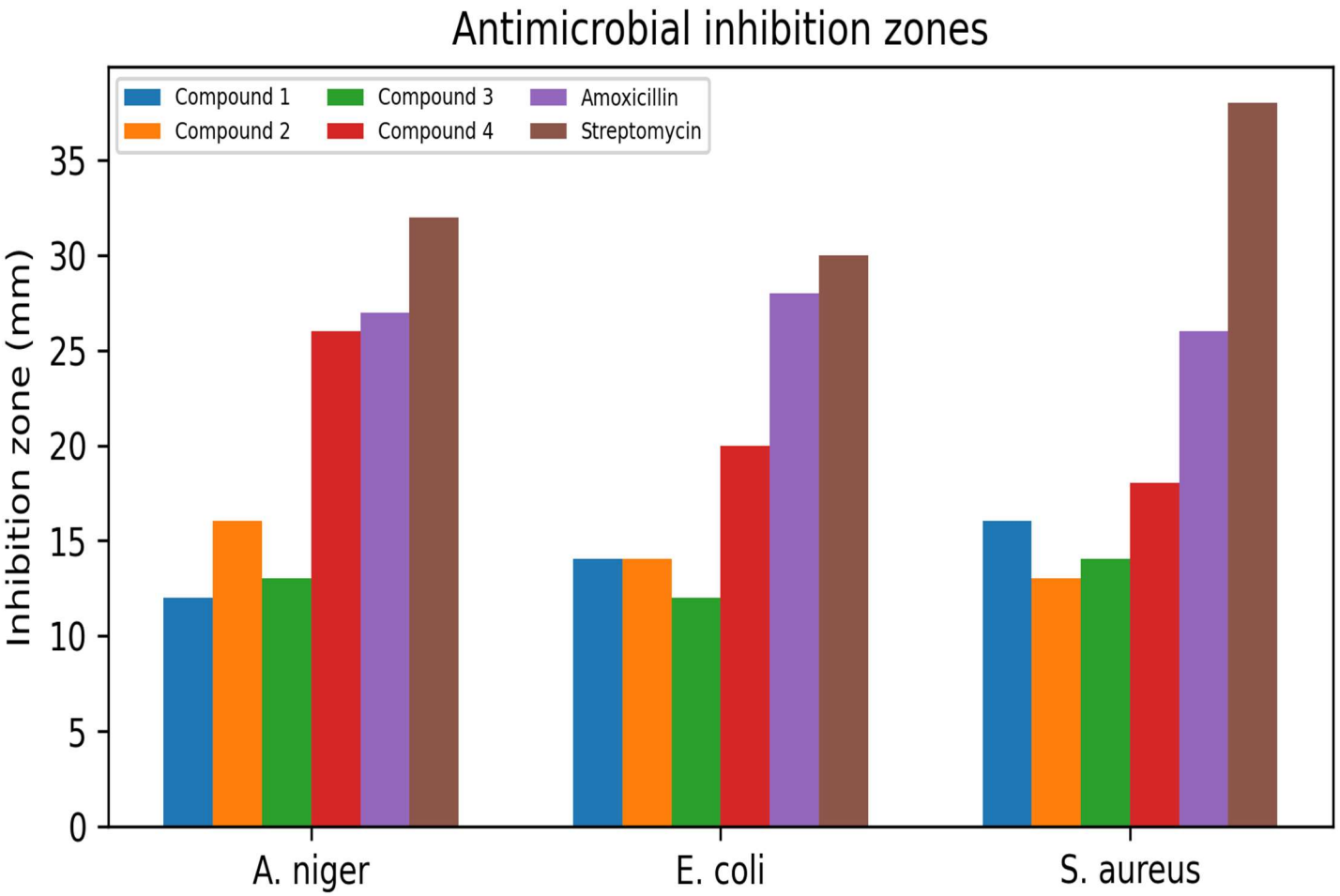


Figure 2. Antimicrobial inhibition-zone comparison.

Compound 4 produced the largest inhibition zones among the synthesized derivatives: 26 mm against A. niger, 20 mm against E. coli and 18 mm against S. aureus.

Minimum inhibitory concentration

Treatment	A. niger	E. coli	S. aureus
Compound 1	400	350	400
Compound 2	450	450	300
Compound 3	450	400	400
Compound 4	250	300	250
Amoxicillin	—	75	50
Streptomycin	—	25	50
Fluconazole	25	—	—

Table 4. Reported MIC values (µg/ml).

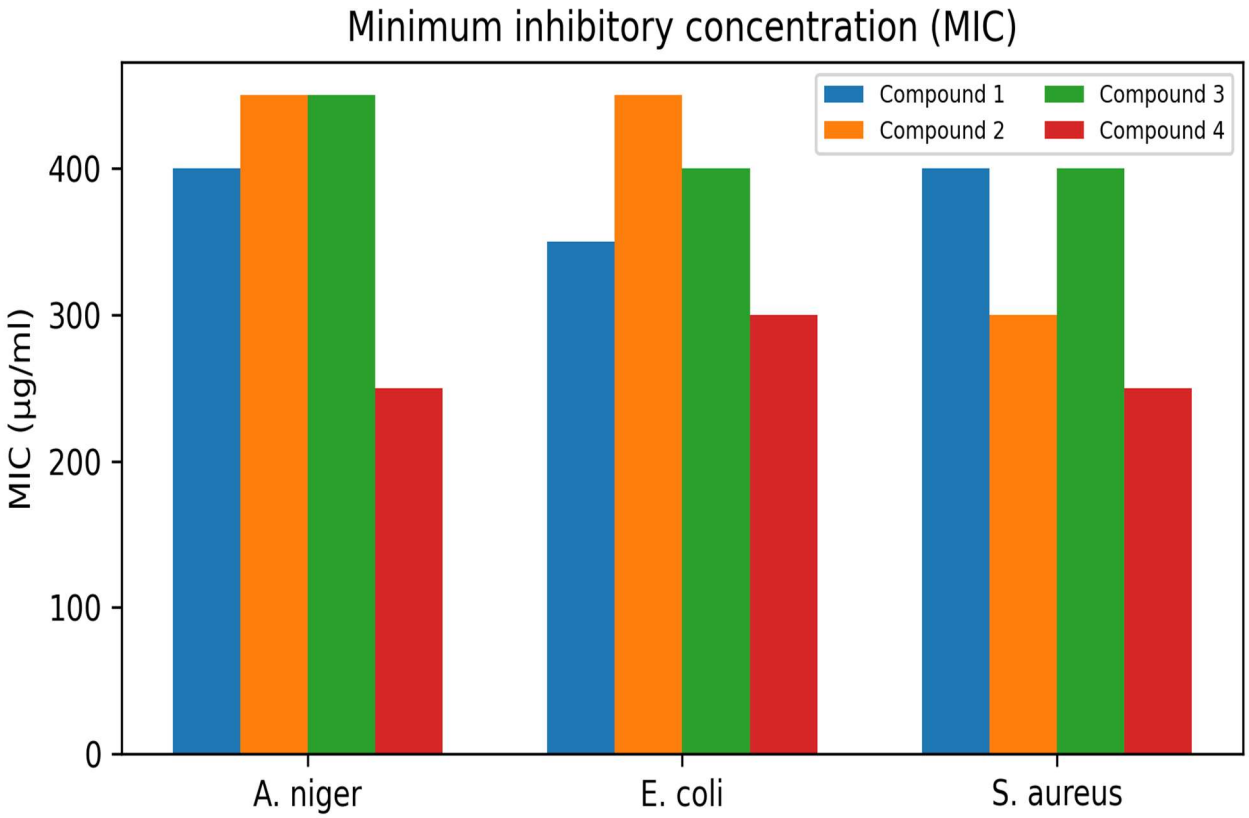


Figure 3. MIC comparison across the reported microbial panel.

Compound 4 showed the lowest MIC values among the synthesized derivatives: 250 µg/ml against A. niger, 300 µg/ml against E. coli and 250 µg/ml against S. aureus. The reference agents generally showed lower MIC values, indicating stronger activity under the reported assay conditions.

Antioxidant activity

Sample	0.1 mM	0.2 mM	0.3 mM	0.5 mM
Compound 1	16.77	19.94	20.88	23.02
Compound 2	23.86	26.00	28.24	28.98
Compound 3	9.88	11.10	13.05	14.54
Compound 4	23.01	25.16	26.74	26.77
Ascorbic acid	33.64	65.89	91.70	94.32

Table 5. DPPH radical-scavenging activity (%).

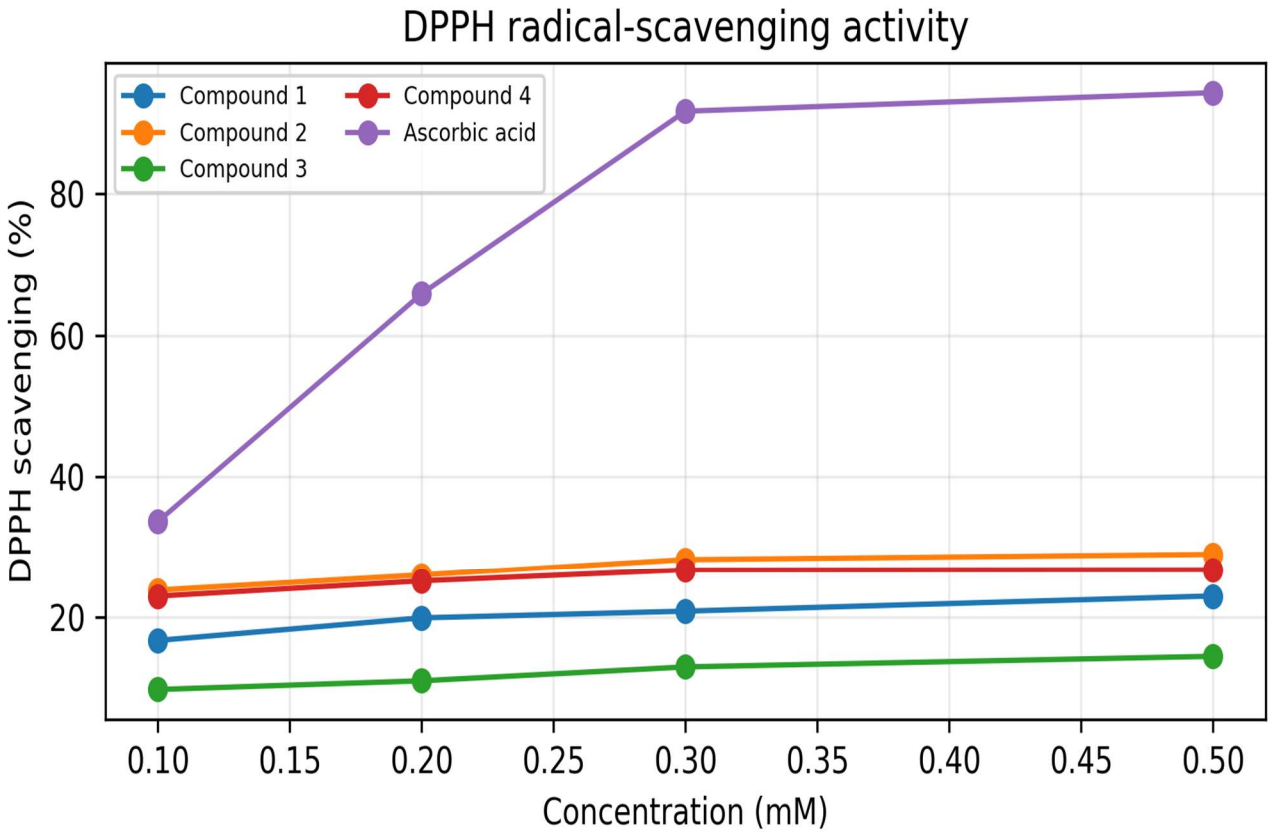


Figure 4. DPPH radical-scavenging profile across concentrations

All synthesized compounds showed increasing scavenging with increasing concentration. At 0.5 mM, Compound 2 showed the highest activity among the synthesized derivatives (28.98%), followed by Compound 4 (26.77%), Compound 1 (23.02%) and Compound 3 (14.54%). Ascorbic acid showed 94.32%.

4.Structure–Activity Relationship and Discussion

The biological data demonstrate assay-dependent differences within the common benzimidazole–pyrazolone framework. Compound 1 was notable in the antifungal evaluation, particularly against C. albicans. Compound 2 showed the highest DPPH scavenging among the synthesized compounds and also showed selected antifungal responses. Compound 4 consistently showed the strongest antimicrobial performance by inhibition-zone and MIC measurements.

The differences may reflect the influence of aromatic substitution on electronic distribution, steric environment, lipophilicity and molecular interactions. The present data, however, are preliminary and do not establish a definitive molecular mechanism or a complete structure–activity relationship.

Comparative interpretation

The chlorinated, nitro, brominated and unsubstituted benzyl groups provide a simple comparative set. The stronger antimicrobial profile of Compound 4 indicates that the unsubstituted benzyl derivative performed best in the tested microbial panel, whereas the strongest antioxidant response was observed with the nitro-substituted Compound 2. These endpoint differences emphasize that biological activity cannot be predicted from a single assay.

Limitations and further work

- Resolve the Compound 3 structure/name discrepancy before submission.
- Confirm compound identity and purity using complete analytical records.
- Expand the analogue series for more reliable SAR interpretation.
- Perform cytotoxicity, selectivity and mechanistic studies.
- Validate promising derivatives in appropriate in-vivo models after safety assessment.

5. Conclusion

Four benzimidazole–pyrazolone derivatives were synthesized, characterized and evaluated in preliminary antifungal, antimicrobial and antioxidant assays. Compound 1 showed the highlighted antifungal response, Compound 2 showed the highest DPPH scavenging among the synthesized derivatives, and Compound 4 showed the strongest antimicrobial profile. The results support continued medicinal-chemistry optimization of the benzimidazole–pyrazolone framework while emphasizing the need for confirmatory analytical, mechanistic, toxicity and in-vivo studies. The antioxidant study demonstrated concentration-dependent scavenging activity for all the synthesized derivatives. Compound 2 exhibited the highest antioxidant activity among the synthesized compounds, reaching 28.98% scavenging at 0.5 mM, followed by Compound 4 with 26.77%. Although the synthesized compounds showed lower activity than the respective standard agents, their measurable biological activities indicate the potential of the benzimidazole scaffold for further optimization. Overall, the study successfully achieved its objective of synthesizing and preliminarily evaluating novel benzimidazole derivatives. Compounds 1, 2, and 4 were identified as particularly promising lead structures based on their biological profiles. Further structural modification, structure–activity relationship studies, molecular docking, mechanism-of-action studies, toxicity evaluation, and in-vivo investigations are recommended to improve their pharmacological potency and therapeutic potential.

6.References

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