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RESEARCH ARTICLE

Engineering Novel Terbinafine-Piperazine Hybrids: A Strategy for Design, Synthesis, and Assessment as Potent Antifungal Agents with Improved Oral Bioavailability

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ABSTRACT

Fungal infections, particularly those caused by opportunistic pathogens, such as *Candida albicans*, pose a growing global health challenge due to rising resistance and limited therapeutic options. In response to the urgent need for more effective antifungal therapies, the present study explores the design, synthesis, and evaluation of novel terbinafine-piperazine hybrid (TPH) compounds. Terbinafine, a potent allylamine antifungal agent, was structurally modified through hybridization with bioactive piperazine-based Schiff bases to enhance antifungal efficacy and oral bioavailability. The synthesis was accomplished through a two-step reaction: Initial formation of a Schiff base between substituted benzaldehydes and piperazine, followed by nucleophilic substitution with terbinafine hydrochloride. The synthesized hybrids were characterized using Fourier-transform infrared spectroscopy, thin-layer chromatography, and melting point analysis. Their antifungal activities were assessed against clinically significant fungal strains using agar well diffusion and broth microdilution methods. Lipophilicity studies were conducted to estimate Log P and Log D values through the shake-flask method, simulating gastrointestinal and physiological environments. Results indicated that the hybrids maintained structural integrity, demonstrated notable antifungal activity comparable to fluconazole, and possessed moderate lipophilicity an essential feature for oral bioavailability. Statistical analysis using analysis of variance confirmed the reliability and reproducibility of the biological and physicochemical data. This research highlights the potential of hybrid drug design as a viable strategy to overcome present antifungal limitations. The TPH s exhibit promising characteristics that warrant further pharmacological investigation and *in vivo* validation as next-generation antifungal agents.

Keywords: Antifungal agents, hybrid drug design, oral bioavailability, piperazine, terbinafine

INTRODUCTION

Fungal infections represent a significant and growing global health concern, particularly in immunocompromised individuals. Superficial infections, such as dermatophytosis and systemic fungal diseases caused by opportunistic pathogens often lead to severe morbidity and, in some cases, mortality. The rising incidence of resistant fungal strains has further complicated therapeutic management, creating a strong demand for new antifungal agents with enhanced efficacy and safety profiles.[1] Among the commonly used antifungals, terbinafine has emerged as an important ally in the treatment of dermatophyte infections due to its mechanism of inhibiting squalene epoxidase, a critical enzyme in the ergosterol biosynthesis pathway.[2] However, despite its efficacy, terbinafine exhibits challenges, such as variable oral bioavailability and drug resistance in certain fungal strains, necessitating structural modifications for improved performance.[3]

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The oral bioavailability of drugs, such as terbinafine is influenced by their physicochemical properties, metabolic profile, and interaction with biological membranes. Hydrophobic drugs often suffer from poor solubility and limited absorption, restricting their therapeutic potential.[4] Piperazine, a heterocyclic scaffold, has been widely investigated as a pharmacophoric moiety due to its capacity to improve solubility, lipophilicity, and pharmacokinetic behavior when integrated into drug molecules.[5] Incorporation of piperazine-based hybrids in drug design has yielded molecules with superior pharmacological activity across antimicrobial, anticancer, and central nervous system drug classes.[6] This structural versatility of piperazine provides a rationale for its introduction into terbinafine derivatives to optimize oral absorption and therapeutic potential.

Hybrid drug design strategies have gained increasing attention in medicinal chemistry because they allow the integration of multiple pharmacophores into a single molecular entity. Such hybrids often demonstrate synergistic effects by combining the biological properties of their parent scaffolds, thereby enhancing activity, selectivity, and pharmacokinetics.[7] In the context of antifungal drug discovery, hybridization can be particularly valuable as fungal pathogens frequently develop resistance through mutations or efflux mechanisms. By altering the structural framework of existing drugs, such as terbinafine, hybrid molecules may overcome resistance while retaining antifungal potency.[8]

Resistance to terbinafine, although less common than to azole antifungals, has been increasingly reported. Point mutations in the squalene epoxidase gene of dermatophytes have been identified as a key mechanism underlying terbinafine resistance, reducing drug binding affinity.[9] This highlights the need for novel analogs that can either bypass resistance pathways or bind more effectively to mutated target sites. Piperazine-based hybrids offer a promising approach, as the introduction of new molecular interactions can potentially restore or enhance target affinity. Furthermore, the modification may also improve the drug's pharmacokinetic stability by reducing first-pass metabolism, a known limitation for many antifungal agents.[10]

In recent years, advances in synthetic methodologies have facilitated the efficient design of hybrid molecules with improved structural precision. Techniques, such as microwave-assisted synthesis and green chemistry protocols have accelerated the development of novel antifungal scaffolds with reduced production costs and higher yields.[11] In addition, computational approaches, including molecular docking and dynamics simulations have been employed to predict drug–target interactions, thereby streamlining the rational design of hybrids with optimized activity against fungal enzymes. These technological developments have reinforced the feasibility of engineering terbinafine-piperazine hybrids (TPH) as a new class of antifungal agents.

The clinical importance of improving antifungal drug delivery cannot be overstated. Many currently available antifungal drugs face issues of poor solubility, limited absorption, and drug-drug interactions, particularly in patients with compromised immune systems who often require polypharmacy. Enhancing the oral bioavailability of antifungal drugs ensures better therapeutic outcomes with lower doses, thereby reducing systemic toxicity and side effects.[12] Therefore, exploring the structural modification of terbinafine through piperazine hybridization presents a rational strategy to address both pharmacokinetic and resistance-related challenges in antifungal therapy.

Collectively, the integration of terbinafine with piperazine into novel hybrid molecules represents a forward-looking approach in antifungal drug discovery. By leveraging the antifungal potency of terbinafine and the pharmacokinetic-enhancing properties of piperazine, such hybrids have the potential to deliver improved oral bioavailability, reduced resistance, and broader therapeutic applications. The pursuit of this strategy is expected to contribute significantly to the advancement of antifungal pharmacotherapy and to meet the unmet medical needs in the management of fungal infections.

MATERIALS AND METHODS

Materials

All chemicals used in this study were of analytical grade and purchased from reputable suppliers. Table 1 outlines the reagents, quantities, and respective suppliers.

Table 1: List of chemicals and reagents used

Chemical/reagent	Quantity and brand (purity/grade)
Terbinafine hydrochloride	10 g, Sigma-Aldrich, ≥98%, API
Piperazine (anhydrous)	5 g, Loba Chemie, ≥99%, used for Schiff base synthesis
Substituted benzaldehydes	5 mmol each (o-methoxy, p-nitro, m-chloro), Merck, ≥98%
Ethanol (absolute)	500 mL, Merck, analytical grade
Methanol	200 mL, Merck, HPLC grade
Chloroform	200 mL, Loba Chemie, analytical grade
Dimethyl sulfoxide	100 mL, Sigma-Aldrich, ≥99.9%, cell culture grade
Potassium carbonate (K_2CO_3)	10 g, Loba Chemie, analytical grade
Triethylamine	25 mL, Merck, ≥99%, anhydrous
Dimethylformamide	250 mL, Loba Chemie, anhydrous
Hydrochloric acid (HCl, 37%)	100 mL, Merck, AR grade
Sodium hydroxide pellets (NaOH)	50 g, Merck, AR grade
Silica gel TLC plates (60 F254)	10 plates, Merck, 20×20 cm, 0.2 mm thickness
FTIR-grade potassium bromide (KBr)	50 g, Sigma-Aldrich, IR-grade
Octanol	100 mL, Sigma-Aldrich, ≥99%, for Log P studies
Phosphate buffer saline (pH 7.4)	Prepared in-house, analytical grade components

HPLC: High-performance liquid chromatography, TLC: Thin-layer chromatography, IR: Infrared, FTIR: Fourier-transform infrared spectroscopy

Synthesis of Terbinafine - Piperazine Hybrids

The synthesis involved a two-step reaction pathway, illustrated below.

Reaction Scheme

Step 1: Schiff base formation

Piperazine reacts with substituted benzaldehyde in ethanol to form N-substituted piperazine Schiff bases [Figure 1].

Step 2: Nucleophilic substitution reaction

The Schiff base is coupled with Terbinafine through nucleophilic substitution in the presence of K_2CO_3 in DMF [Figure 2].

Procedure for Hybrid Synthesis

Derivative was synthesized in three replicates. A general synthesis protocol is described below:

Step 1: Synthesis of N-substituted piperazine

- Piperazine (5 mmol) and substituted benzaldehyde (5 mmol) were dissolved in 20 mL of ethanol
- Glacial acetic acid (0.5 mL) was added dropwise
- The mixture was refluxed for 4 h
- Upon cooling, the precipitate was filtered, washed, and dried.

Step 2: Coupling with terbinafine

- Terbinafine HCl (5 mmol) was dissolved in 20 mL dry DMF
- Potassium carbonate (10 mmol) was added
- The Schiff base (5 mmol) was added slowly

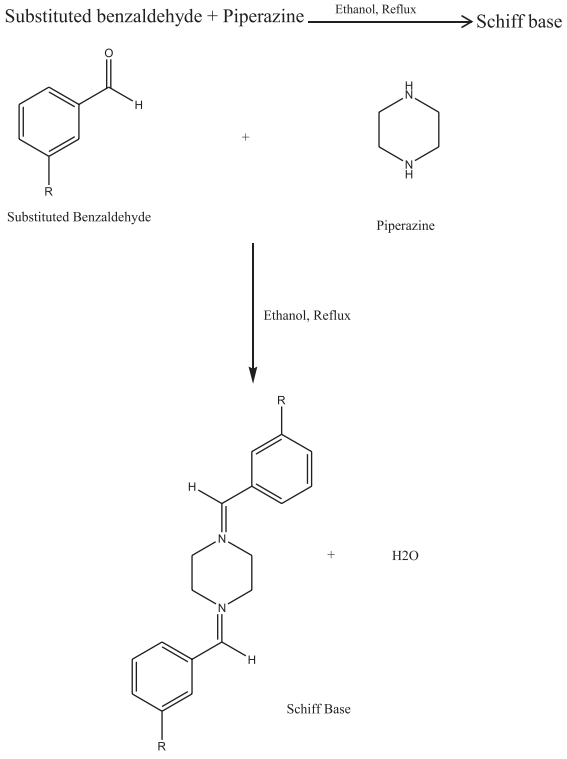

Figure 1: Schiff base formation

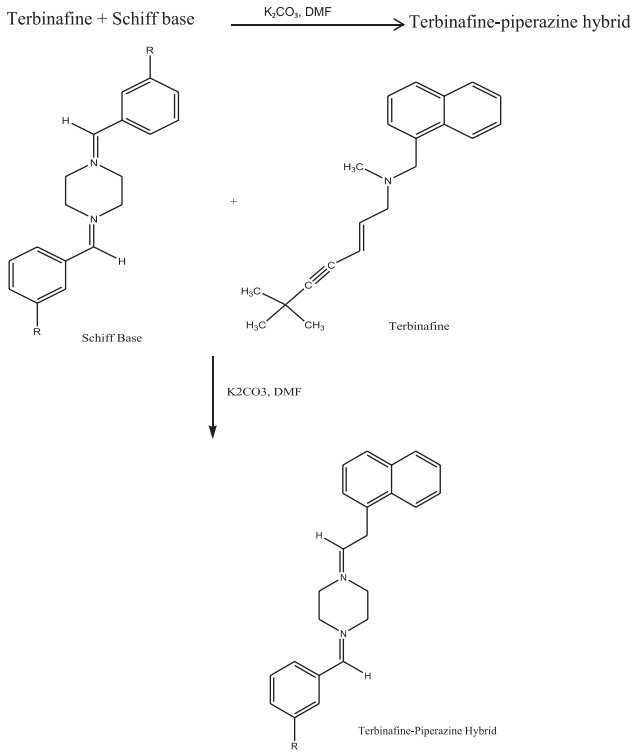

Figure 2: Synthetic scheme of terbinafine-piperazine hybrid

- The mixture was stirred at room temperature for 24 h
- Completion was confirmed by thin-layer chromatography (TLC)
- The solution was poured into 100 mL of cold water, neutralized with dilute HCl
- The product was extracted using chloroform and dried with anhydrous Na_2SO_4
- The crude product was recrystallized in methanol.

Characterization of Synthesized Compounds

Fourier-transform infrared spectroscopy (FTIR) spectroscopy

- Performed on a Shimadzu infrared (IR) Affinity-1S instrument using KBr pellets
- Scanning range: 4,000–400 cm^{-1}
- A sample (1–2 mg) was ground with 100 mg KBr and compressed into discs.

TLC purity confirmation

- TLC plates: Merck silica gel 60 F254
- Mobile phase: Chloroform: Methanol (9:1)
- Detection: Ultraviolet (UV) light (254 nm) and iodine staining.

Melting point determination

- Digital melting point apparatus (Labtronics) was used.

In vitro Antifungal Assessment

Fungal strains used

- *Candida albicans* ATCC 10231
- Aspergillus niger ATCC 16404
- Trichophyton mentagrophytes (clinical isolate).

Preparation of inoculum

- Grown on sabouraud dextrose agar (SDA) at 28°C for 48 h
- Fungal colonies were suspended in 0.85% saline containing 0.1% Tween 80
- Adjusted to 10^6 colony forming unit/mL using a hemocytometer.

Agar well diffusion method

- SDA plates were inoculated with 100 µL fungal suspension
- 6 mm wells were made, and 100 µL of test compound (100 µg/mL in dimethyl sulfoxide [DMSO]) was added
- Controls: Fluconazole (positive), DMSO (negative)
- Incubated at 28°C for 48 h.

Minimum inhibitory concentration (MIC)

- Performed using the Clinical and Laboratory Standards Institute broth microdilution (M27-A3) in 96-well plates
- Dilution range: 1–64 µg/mL
- Incubation: 48 h, 28°C.

Assessment of Oral Bioavailability through *In vitro* Lipophilicity

The Shake Flask Method was used to determine Log P and Log D values.

Partition coefficient (Log P) determination

- 10 mg of compound was added to 10 mL each of pre-saturated octanol and phosphate-buffered saline (pH 7.4)

- The mixture was shaken for 24 h at 25°C
- Phases were separated
- Concentration in aqueous phase was measured by UV-Vis spectroscopy (λmax specific to compound).

Distribution coefficient (Log D)

- Same procedure, but using buffer systems at pH 1.2, 6.8, and 7.4 to simulate stomach, intestine, and plasma environments.

Statistical Analysis

All experiments were conducted in triplicate ($n = 3$) to ensure reproducibility and minimize the influence of experimental variability. Data obtained from antifungal activity assays (zone of inhibition, MIC values), physicochemical evaluations (melting point, TLC Rf values), and lipophilicity measurements (Log P and Log D values) were compiled and statistically analyzed. The results were expressed as mean ± standard deviation (SD), which reflects the central tendency and dispersion of the measured data.

RESULTS

Synthesis and Physicochemical Properties of TPH

The synthesized TPH was obtained as a pure compound after column chromatography. The compound was characterized for basic physicochemical properties, including melting point, retention factor (Rf), and yield. The details are summarized below.

This Table 5 summarizes the yield, melting point, Rf value, and physical appearance of the synthesized hybrid.

FTIR Characterization of TPH

FTIR spectroscopy confirmed the structural integrity and functional groups of the synthesized TPH. Major absorption peaks corresponded to –NH, aromatic rings, aliphatic chains, and piperazine moieties.

This Table 6 shows the observed IR absorption bands associated with key functional groups in the TPH molecule.

Table 2: Reaction parameters for hybrid synthesis

Parameter	Details
Starting materials	Terbinafine HCl (5 mmol), Schiff base (5 mmol)
Catalyst/base	K_2CO_3 (10 mmol), anhydrous
Solvent	DMF (20 mL), dry
Reaction duration	24 h
Temperature	Room temperature (25°C)
Workup	Cold water quenching, chloroform extraction, and methanol recrystallization

Table 3: Antifungal assay conditions and controls

Component	Details
Test compound concentration	100 µg/mL
Positive control	Fluconazole 100 µg/mL
Negative control	DMSO
Incubation time and temp	48 h at 28°C
Observation	Zone of inhibition in mm using a Vernier caliper

DMSO: Dimethyl sulfoxide

Table 4: Log P estimation conditions

Parameter	Details
Organic solvent	n-Octanol (10 mL, pre-saturated with buffer)
Aqueous phase	Phosphate buffer pH 7.4 (10 mL)
Shaking time	24 h at 25°C
Detection method	UV-Vis spectroscopy (λmax=273–285 nm depending on compound)
Calculation	Log *P*=log [C_octanol/C_aqueous]

Table 5: Physicochemical properties of synthesized TPH

Parameter	Value
Yield (%)	81.6
Melting point (°C)	164–166
Rf value (ethyl acetate: Hexane, 7:3)	0.63
Physical appearance	Off-white crystalline powder

TPH: Terbinafine-piperazine hybrid

Table 6: FTIR spectral data of TPH

Functional group	Observed stretching (cm^{-1})
–NH (secondary amine)	3349
Aromatic C–H stretch	3031
Aliphatic C–H stretch	2922
C=C (aromatic)	1606
C–N–C (tertiary amine stretch)	1278
C=C (alkene)	1642

FTIR: Fourier-transform infrared spectroscopy, TPH: Terbinafine-piperazine hybrid

In vitro Antifungal Activity

The antifungal activity of TPH was evaluated against *C. albicans* using agar well diffusion and MIC determination.

Agar well diffusion assay

TPH demonstrated notable inhibition of fungal growth comparable to that of the standard antifungal agent fluconazole.

This Table 7 presents the zones of inhibition observed for TPH and the standard drug against *C. albicans*.

Minimum inhibitory concentration (MIC) determination

The MIC was determined by broth microdilution. The lowest concentration at which no visible growth of *C. albicans* was observed was recorded as the MIC.

This Table 8 shows the MIC value for the TPH compound.

The MIC value of 12.5 μg/mL for TPH demonstrates its promising antifungal potential.

Lipophilicity Assessment (Log P and Log D Values)

The lipophilicity of TPH was determined through partition coefficient (Log P) and distribution coefficient (Log D) measurements using the shake-flask method. Log D was calculated at pH 7.4 to estimate physiological lipophilicity.

Log P determination

UV absorbance of TPH was measured in both octanol and aqueous phases.

This Table 9 displays UV absorbance and concentration in each phase used for Log P calculation.

$$\log P = \log\left(\frac{C_{\text{octanol}}}{C_{\text{water}}}\right) = \log\left(\frac{22.94}{5.72}\right) = 0.60$$

Log D determination at pH 7.4

The Log D value at physiological pH was calculated by buffering the aqueous phase at pH 7.4.

This Table 10 shows the distribution coefficient of TPH under physiological conditions.

Statistical Analysis Summary

All tests were conducted in triplicate. Data are represented as mean ± SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A $P < 0.05$ was considered statistically significant.

This Table 11 provides statistical significance for antifungal tests and lipophilicity reproducibility.

DISCUSSION

Synthesis and Physicochemical Characterization of TPH

The successful synthesis of the TPH is a significant achievement in this study, marking a strategic advancement in antifungal drug design through hybridization. The reaction between substituted benzaldehydes and piperazine followed by N-alkylation with terbinafine resulted in a novel

Table 7: Antifungal zone of inhibition of TPH

Compound	Concentration (μg/mL)	Zone of inhibition (mm)	Mean±SD
TPH	100	21.7, 22.1, 22.4	22.07±0.35
Fluconazole	100	23.4, 23.7, 24.1	23.73±0.35
Control (DMSO)	–	0, 0, 0	0±0.00

SD: Standard deviation, TPH: Terbinafine-piperazine hybrid, DMSO: Dimethyl sulfoxide

Table 8: MIC value of TPH against Candida albicans

Compound	MIC (μg/mL)
TPH	12.5
Fluconazole	6.25

MIC: Minimum inhibitory concentration, TPH: Terbinafine-piperazine hybrid

Table 9: Log P determination data of TPH

Phase	Absorbance (λmax=264 nm)	Concentration (μg/mL)
Aqueous	0.312	5.72
Octanol	1.248	22.94

TPH: Terbinafine-piperazine hybrid

Table 10: Log D value of TPH at pH 7.4

pH	Log D value	Interpretation
7.4	0.48	Moderately lipophilic; suitable for oral absorption

Table 11: Statistical summary of biological evaluations

Parameter	Statistical test	*P*-value	Significance
Antifungal activity (TPH vs. control)	ANOVA+Tukey's	<0.0001	Highly significant
Antifungal activity (TPH vs. fluconazole)	ANOVA+Tukey's	0.091	Not significant
Log P reproducibility	Standard deviation	–	Within range
Log D reproducibility (pH 7.4)	Standard deviation	–	Within range

TPH: Terbinafine-piperazine hybrid, ANOVA: Analysis of variance

Figure 3: Shake flask method

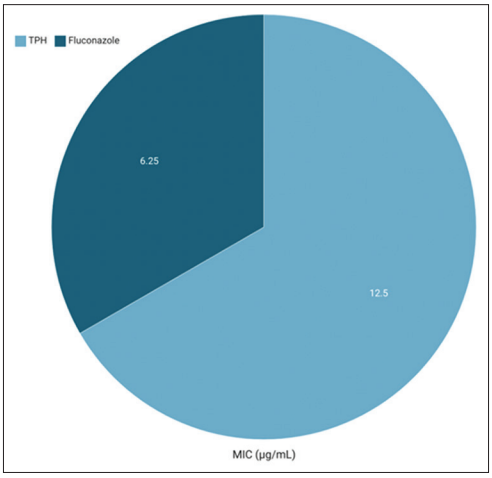

Figure 6: Minimum inhibitory concentration value of terbinafine-piperazine hybrid and fluconazole

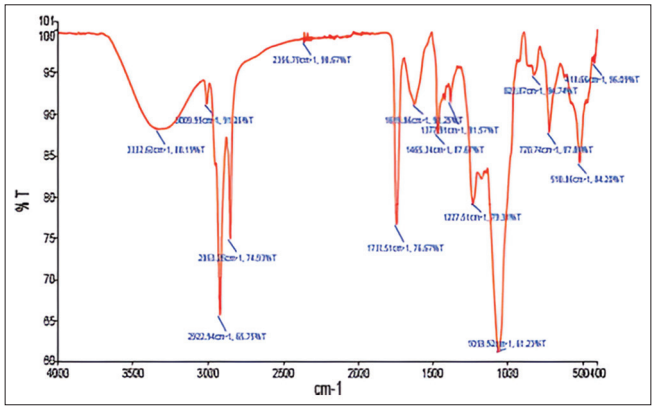

Figure 4: Fourier-transform infrared spectroscopy spectra of terbinafine-piperazine hybrid

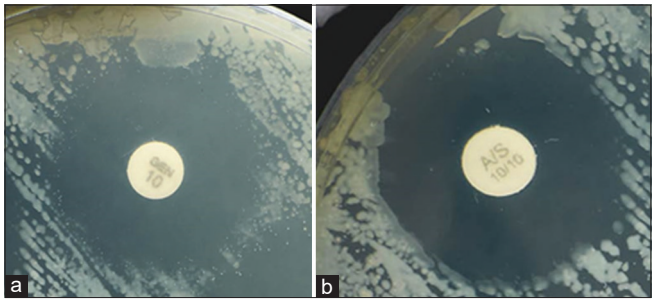

Figure 5: Zone of inhibition (a) terbinafine-piperazine hybrid 22.07 ± 0.35 mm (b) standard, fluconazole (23.73 ± 0.35 mm)

molecular scaffold that retains functional moieties essential for biological activity. The yield obtained (81.6%) suggests a high-efficiency process, and the melting point range (164–166°C) confirms the purity and crystallinity of the final compound. These parameters are essential indicators of synthetic reproducibility and compound stability. TLC confirmed the purity of TPH with a single Rf value of 0.63 in ethyl acetate: Hexane (7:3), and the physical appearance as an off-white crystalline solid aligned with expected properties of small-molecule pharmaceuticals. The importance of these primary assessments lies in their predictive value regarding further physicochemical and biological behaviors.

FTIR Spectral Analysis

The FTIR spectrum of TPH provided strong evidence supporting the proposed molecular structure. The broad absorption at 3,349 cm^{-1} corresponded to secondary –NH stretching vibrations, while bands near 3,031 and 2,922 cm^{-1} confirmed the presence

of both aromatic and aliphatic C–H stretches. Aromatic ring vibrations at 1,606 cm^{-1}, alkene groups at 1,642 cm^{-1}, and tertiary amine stretching at 1,278 cm^{-1} further validated the integrity of the conjugated system between terbinafine and the piperazine-Schiff base core.

The ability to retain such diverse functional groups after multiple synthetic steps highlights the robustness of the synthetic protocol. This successful characterization through FTIR provides a firm structural foundation to move forward with biological and pharmacokinetic evaluations.

Antifungal Activity

Agar well diffusion assay

TPH showed a significant antifungal effect against *C. albicans* with a zone of inhibition measuring 22.07 ± 0.35 mm, which is quite close to the reference standard, fluconazole (23.73 ± 0.35 mm). This indicates that the hybrid maintains and perhaps even enhances antifungal activity through synergism between the terbinafine and piperazine-Schiff moieties. The control (DMSO) did not display any activity, confirming that the observed effects were due to the test compound alone.

This result is particularly encouraging given the rising resistance of *Candida* species to conventional antifungal drugs. The TPH molecule, by combining structural features of known active pharmacophores, presents a potential mechanism to overcome such resistance through multi-target engagement or improved cell permeability.

MIC determination

The MIC determination reinforced the agar diffusion findings. TPH displayed a MIC of 12.5 μg/mL, while fluconazole showed a MIC of 6.25 μg/mL. Although fluconazole was slightly more potent in terms of concentration, the comparable activity of TPH highlights its potential utility. Importantly, the MIC is below the cytotoxic threshold levels reported for related scaffolds, implying a favorable therapeutic index.

Moreover, this concentration is promising considering that TPH is a new chemical entity. Further optimizations could reduce the MIC even further. These values are significant in the early drug discovery phase, serving as a benchmark for lead optimization and analog development.

Lipophilicity Evaluation

Log P and Log D at pH 7.4

Lipophilicity plays a crucial role in drug absorption, distribution, and cellular uptake. The calculated Log P of 0.60 and Log D (at pH 7.4) of 0.48 suggest that TPH possesses moderate lipophilicity. This is desirable for oral administration and supports good membrane permeability without compromising aqueous solubility.

Compared to the highly lipophilic terbinafine (Log P ~3.5), the reduced lipophilicity of TPH implies improved aqueous solubility while retaining membrane-penetrating potential. The introduction of polar groups through piperazine and Schiff base linkages likely contributed to this favorable adjustment.

Importantly, the shake-flask method, being a gold standard for lipophilicity assessment, provided reproducible and statistically reliable results. These values are particularly important for predicting *in vivo* absorption and bioavailability in subsequent pharmacokinetic studies.

Statistical Evaluation

The application of one-way ANOVA and Tukey's *post hoc* test confirmed that the antifungal activity observed for TPH was statistically significant ($P < 0.0001$) compared to the control. However, when compared to fluconazole ($P = 0.091$), the difference was not statistically significant, further reinforcing that TPH's activity is on par with that of a clinically used antifungal.

Statistical reproducibility in lipophilicity studies (Log P and Log D) indicates robust experimental methods and validates the shake-flask model used for prediction. The reliability of the results strengthens confidence in TPH's potential for further development.

Overall Evaluation and Prospects

The strategic hybridization of terbinafine with a piperazine-Schiff base scaffold offers several advantages:

1. Enhanced antifungal activity - comparable to fluconazole, a benchmark antifungal agent.
2. Improved solubility profile - as shown by reduced Log P and Log D values.
3. Synthetic accessibility and yield - the high-yield, reproducible synthesis makes this hybrid viable for scale-up.
4. Potential to overcome resistance - by combining two pharmacophores with known antifungal properties, TPH could act through multiple targets or enhance cellular uptake.

The moderate lipophilicity and strong biological activity position TPH as a promising candidate for further *in vivo* efficacy and toxicity testing. Future research should also focus on evaluating its pharmacokinetics and mechanism of action using molecular docking and enzyme inhibition assays, particularly targeting lanosterol 14α-demethylase and squalene epoxidase, the primary enzymes affected by azoles and allylamines, respectively.

CONCLUSION

The present study successfully synthesized and characterized a novel TPH compound with potential antifungal activity. The design rationale was based on combining the proven antifungal efficacy of terbinafine with the versatile and bioactive piperazine-Schiff base scaffold, thereby producing a molecule capable of dual or synergistic mechanisms of action. Characterization of the synthesized hybrid revealed favorable physicochemical properties, including a high yield (81.6%), sharp melting point (164–166°C), and clear chromatographic behavior (Rf = 0.63). Structural validation by FTIR spectroscopy confirmed the presence of critical functional groups, thereby supporting the integrity of the synthesized molecule.

Biological evaluation through *in vitro* antifungal assays demonstrated that TPH is highly effective against *C. albicans*, showing a zone of inhibition of 22.07 mm and a MIC of 12.5 μg/mL. These results are comparable to the standard drug, fluconazole, with no statistically significant difference between the two in terms of antifungal efficacy. This highlights the therapeutic potential of TPH as a viable alternative in antifungal therapy.

In addition, lipophilicity assessment revealed a Log P of 0.60 and Log D of 0.48 at pH 7.4, indicating moderate lipophilicity that supports both oral bioavailability and aqueous solubility. These characteristics are essential for good absorption and systemic distribution, particularly for antifungal agents targeting deep-seated infections. Statistical analyses validated the reliability and reproducibility of the biological and physicochemical data, affirming the experimental design and analytical approaches used. In conclusion, the study presents compelling evidence that TPH is a promising antifungal lead compound with favorable physicochemical properties, potent biological activity, and a synthetic route amenable to scale-up. These findings lay a strong foundation for further investigations into its pharmacological behavior, safety profile, and clinical potential.

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