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Design, Synthesis and Characterization of 5-(2-Bromo-5-Fluorophenyl)-3-(1h-Pyrrol-2-Yl)-4,5-Dihydro-1h-Pyrazole as Antifungal Agent

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Abstract
This study successfully synthesized a novel compound (5-(2-bromo-5-fluorophenyl)-3-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazole (**6**), from an α, β-unsaturated carbonyl compound (**5**). The compound was characterized using various spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Its antifungal potential was evaluated against three fungal strains including *Candida tropicalis, Candida parapsilosis, and Candida albicans*. The results indicated that compound (**6**) exhibited moderate antifungal activity. Furthermore, molecular descriptors and ADMET profiling were employed in an *in-silico* simulation to assess the compound's drug-likeness. This study thus presents a valuable strategy for developing more effective antifungal agents.

Keywords: Synthesis, ADMET, Drug-likeness, Antifungal, Characterization.

INTRODUCTION

Fungal infections have risen significantly in prevalence over the past three decades, presenting a major challenge in the field of infectious diseases, particularly in the development of novel treatments for systemic mycoses. The urgent need for new antifungal therapies with innovative mechanisms of action is driven by the increasing resistance of fungi to existing antifungal agents and the growing population of immunocompromised individuals, such as those with HIV or neutropenia. A key barrier to the development of safe and effective antifungals is the rapid emergence of resistance, compounded by the biochemical similarities between human cells and fungi, which complicates the design of drugs with selective activity[1-2]. Heterocyclic compounds are valuable in the field of medicinal chemistry due to their diverse biological potential. [3][4]. The ring of the five-membered heterocyclic molecule pyrazoline contains two adjacent nitrogen atoms [5]. It contains just one endocyclic double bond and is alkaline by nature [6]. Nonetheless, pyrazole is present in the structures of certain alkaloids, such as sesquiterpene pyrazolines, piperidine alkaloids, and pyrazoline alkaloids [4]. Black pepper (*Piper nigrum*) and other plants in the Piperaceae family naturally contain pyrazolines, also referred to as piperidine alkaloids [7][8]. The most well-known heterocyclic compounds are pyrazoline derivatives, which have intriguing biological properties. [4]. which consist of HIV reverse transcriptase inhibitors that are non-nucleoside antagonists of neurotensin receptors with analgesic effects and non-steroidal mineralocorticoids[5], antiviral[6], antitumor[7][8], antimicrobial[9][10], antitubercular[11], antimalarial[12], anti-amoebic[13], antifungal[14], antidiabetic[15], anti-inflammatory [16], anticancer[17]. Pyrazolines have consequently recently been required as a scaffold for a variety of drugs with a wide range of actions.[18]. Some of the biological activities associated with them include, antimicrobial[19], antioxidant[20], anti-inflamatory[21], antitubucular[22], anticancer[23], cardioprotective, antidiabetic[24], antiviral[25], anti-ageing[26], antiallergic[27], and hepatoprotective[28]. It is present in several commercially available drugs in a variety of categories., including morazone (**1**) and antipyrine (**2**) [29][30] (**Figure 1**).

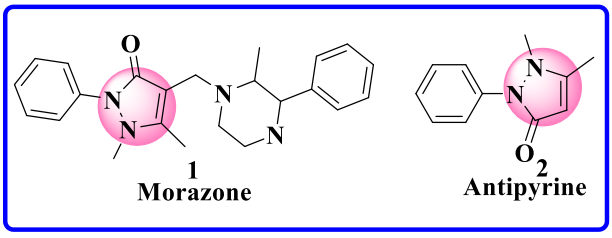

Fig. 1. Pyrazoline based compounds[31][32]

This research investigated the antifungal properties of the compound 5-(2-Bromo-5-Fluorophenyl)-3-(1H-Pyrrol-2-Yl)-4,5-Dihydro-1H-Pyrazole (6). The compound was characterized using a variety of spectroscopic techniques, including

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^{1}H NMR, ^{13}C NMR, and mass spectrometry. Its antifungal efficacy was further evaluated against three fungal species including *Candida tropicalis*, *Candida parapsilosis*, and *Candida albicans*. Its cytotoxicity was assessed with VERO cells. Additionally, molecular descriptors and ADMET profiling were employed in an *in-silico* simulation to assess the compound's drug-likeness.

EXPERIMENTAL

Material and methods

All solvents and chemical reagents from Merck and did not further purify them during use. Fourier transform infrared spectroscopy (FTIR) analysis using a PerkinElmer Nicolet 6700 FTIR spectrometer. We used a Bruker Avance 400 MHz UltrashieldTM spectrometer for ^{1}H and ^{13}C NMR and dissolved the sample in DMSO-d^6. The ESI-MS spectra using ThermoElectron Corporation's ion trap LCQ Advantage Max mass spectrometry. The Stuart SMP10 melting point apparatus to ascertain the melting points of the synthesised compounds.

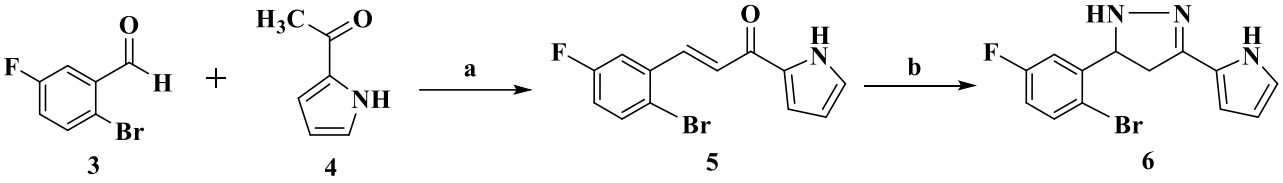

Fig. 2. Scheme for the Synthesis of 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)**

Spectral characterization

General procedure for the synthesis of 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole (6)

Ethanol (25 mL) was used to dissolve the chalcone (0.001 mol) in the MeOH (10 mL), and hydrazine hydrate was added and the mixture stirred for 2-3 h [33][34]. We monitored the progress of the reaction using TLC [35]. On completion, the crude mixture was poured into ice-cold water, and the separated precipitate was filtered off. The obtained solid compound (6) was washed by distilled water and n-hexane to obtain pure compounds (**Figure 2**).

5-(2-bromo-5-fluorophenyl)-3-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazole (6)

Light brown solid, yield 63%, R_f value: 0.60 (10% MeOH : $CHCl_3$), mp 124-125 °C, FTIR (KBr) ν_{max}: 3089(C-H aromatic), 1384(C-H) 1609(C=N), 1609(-C=C- aromatic), 1462(C=C), 3308(N-H), 1263(C-N) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 2.63(1H, dd, J = 9.5, 16.0Hz), 3.51(1H, dd, J = 10.5, 16.0Hz), 4.92-(1H, brt), 6.04(1H, brs), 6.24(1H, brs), 6.79(1H, brs), 7.12(1H, m), 7.29(1H, brs), 7.36(1H, m), 7.68(1H, m), 11.22(1H, s); ^{13}C-NMR (400 MHz, DMSO-d_6) δ_c: 40.9, 62.4, 108.9, 109.6, 115.4(d, $J_{C\text{-}F}$ = 24.2 Hz), 116.6(d, $J_{C\text{-}F}$ = 22.6 Hz),117.0, 120.9, 125.7, 134.8(d, $J_{C\text{-}F}$ = 8.0 Hz), 144.0, 145.4(d, $J_{C\text{-}F}$ = 6.8Hz), 163.2(d, $J_{C\text{-}F}$ = 142.6Hz) ; ES-MS (*m/z*): 308 $[M]^+$, 310 $[M+2]^+$, calculated for $C_{13}H_{11}BrFN_3$.

Biological Evaluation

In vitro antifungal activity

The synthesised compound was evaluated for its *in vitro* antifungal activity against *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. Fungal cultures in 96-well plates were conducted using RPMI 1640 Medium buffered with MOPS [3-(N-morpholino propanesulphonic acid)]. Susceptibility testing was conducted in accordance with the guidelines established by the Clinical and Laboratory Standard Institute (CLSI). The highest concentration evaluated was 50 µg/mL, and the inoculum load in each test ranged from 1 to 5×10^3 cells. Prior to the visual determination of minimal inhibitory concentrations (MIC), the plates were incubated at 35 ± 1°C for a duration of 24 to 48 hours for yeasts and 72 to 96 hours for mycelial fungi[36][37].

Molecular descriptors (MDs) analysis

The Molinspiration Property Engine v2022.08 was utilised in this study to calculate the molecular physicochemical properties of the synthesised compounds. The percentage of absorption (% ABS) was calculated using the formula % ABS = 109 − (0.345 × TPSA). The topological polar surface area (TPSA), molecular weight (MW), partition coefficient (log P), number of rotatable bonds (RB), hydrogen bond acceptor sites (ON), and hydrogen bond donors (OHNH) were identified. Recently, researchers introduced various MD's rules to assess the drug-likeness of chemical scaffolds, with Lipinski's rule of five (Ro5) achieving the highest level of acceptance. This rule indicates that drug design and development (DDD) significantly mitigates misleading outcomes [38].

ADMET analysis

In this study, utilised the ADME SAR tool to evaluate the ADMET properties of the synthesised compounds. This server frequently updates the datasets available for structure-based searches aimed at identifying ADMET properties [56], [57].

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RESULTS AND DISCUSSION

Chemistry

The synthesis of 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)** reacting α, β-unsaturated carbonyl compounds **(5)** with hydrazine or its derivatives to form the pyrazoline ring using sodium hydroxide in methanol as base, at room temperature (**Fig. 2**). All the synthesized compounds were characterized using NMR, MS, UV and FTIR spectral techniques.

Biological Evaluation

In vitro antifungal activity

The synthesized 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)** were subjected to *in vitro* antifungal assay against three strains of fungus, namely *Candida albicans, Candida parapsilosis and Candida tropicalis*, utilizing the Standard Broth Micro dilution method CLSI guidelines[36]. The obtained results were then compared, in terms of minimum inhibitory concentration (MIC, 50 µg/mL), with those of the standard drugs Fluconazole. as displayed in compounds **(6)** displayed significant antifungal activity with MIC value of 25, 25 and 50 µg/mL against *C. albicans, C. parapsilosis and C. Tropicalis*, respectively and standard fluconazole showed the the activity with MIC value of 0.25, 2 and 1 µg/mL against *C. albicans, C. parapsilosis and C. Tropicalis*, respectively. Showed moderate activity with MIC value of 25-50 µg/mL. *Candida* is the most common fungal pathogen responsible for mucosal, cutaneous and systemic infections in humans of different age groups. The three well known species of *Candida* accounting for > 75% of infections are *C. albicans, C. parapsilosis and C. Tropicalis*. The virulence of these fungal infections and the risk of increasing resistance towards available marketed drugs will always boost the finding of effective treatment.

In silico drug likeness and ADMET study

Many hits in clinical trials fail due to a poor pharmacokinetic profile. Thus, there is no denying the significance of absorption, distribution, metabolism, and excretion (ADME) research for the creation of novel medications.[58]. Failure of the hit molecule in the late-stage drug development increases the financial burden on the funding agencies, as well brings wastage of uncompensated time. Hence, the *in silico* ADME shows to be cost and time-effective approach in the drug discovery method which eliminates the unfit candidates in the early stages. With this aim, the synthesis of 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)** were subjected to an *in-silico* study using the Molinspiration web tool and the values thus obtained are summarised in **Table 1**. For most of the compounds the topological polar surface area (TPSA) was between 40.18 $Å^2$ which means that they have good cellular absorption, n-ROTB is ≥3 which represented good molecular flexibility, molecular volume (MV) ranged 221.71 showing less steric hinderance and more cellular transportation, logP values which is a measure of molecular hydrophobicity was found <5 denoted good absorption, bioavailability, hydrophobic drug-receptor interactions. Besides this, Lipinski's rule was followed in majority of the molecules. Thus, from the calculated ADME parameters, the series of synthetic compounds showed a good absorption profile, which could be interpreted. 107.56% with drug-likeness properties (**Table 1-2**).

Table 1. In- *silico* drug lkeness of synthesized compounds 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)** and standard drug (fluconazole)

Compound	% ABS	TPSA ($Å^2$)	n-ROTB	MV	MW	Mi LogP	n-ON acceptors	n-OHNH donors	Lipinski's violation
	-	-	-	-	**<500**	**<5**	**<10**	**<5**	**≤1**
6	107.56	40.18	2	221.71	308.15	3.37	3	2	0

n-ON acceptors – number of hydrogen bond acceptors, n-ROTB – number of rotatable bonds, n- OHNH donors – number of hydrogen bond donor.

Table 2. Display the relative ADMET profiles of Synthesized 5-(2-bromo-5-fluorophenyl)-3-(1h-pyrrol-2-yl)-4,5-dihydro-1h-pyrazole **(6)** and standard drug (fluconazole).

Comp.	BBB		Caco2		HIA		HOB		Carcino		EI		AMES		Biode		AOT
	Val	Prob	Val	Prob	Val	Prob	Val	Prob	Val	Prob	Val	Prob	Val	Prob	Val	Prob	Prob
6	+	0.93	+	0.52	+	1.00	-	0.80	-	0.67	-	0.98	-	0.66	-	1.00	0.50

CONCLUSION

This study successfully synthesized a novel compound (5-(2-bromo-5-fluorophenyl)-3-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazole (**6**). The synthesized compound was tested against three fungal stains including *Candida albicans, Candida parapsilosis,* and *Candida tropicalis*. Compound (**6**) demonstrated selective antifungal activity against these strains exhibiting inhibitory effects at concentrations ranging from 25 to 50 µg/mL. Using the admetSAR tool, an *in-silico* assessment of compound (**6**) was taking place. Drug-likeness and ADMET properties revealed that it possesses good ADMET characteristics. Therefore, this study presents a valuable strategy for the development of more effective antifungal agents.

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Conflict of interest
The authors declare no competing interests.

Author contributions
MBL, *ARK* and *IA* planned and supervised the experiments. *CSY* synthesized, characterized compounds and prepare the initial draft of the manuscript, while *VKV & ZRW* assisted in the manuscript's writing and review. *AK* carried out the experiments involving antifungal activities. *IA* supervised the experiments in chemistry. All authors discussed the results and contributed to the final manuscript.

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