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항칸다디아 활성이 우수한 *bis* acetylated hybrid pyrazoles의 합성 연구

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Novel Synthesis of *bis* Acetylated Hybrid Pyrazoles as Potent Anticandidiasis Agents

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요 약. *Bis* acetylated hybrid pyrazoles 을 합성하여 이들 화합물에 대해 녹는점, 원소분석, MS, FT-IR, one-dimensional ^{1}H,- 및 ^{13}C-NMR로 분석하였다. 합성한 화합물들에 대해 *in vitro* 항균활성을 *Candida sp.* namely *Candida albicans, Candida glabrata, Candida parapsilosis, Candida dubliniensis* 및 *Candida tropicali* 균에 대해 수행하였다. Pyrazoles의 페닐고리에 작용기(-CH_3, -OCH_3, -F, -Cl, 및 Br)가 있는 화합물은 *Candida* species에 대해서 강한 활성을 나타내었다.
주제어: 피라졸, 아실화 반응, 스펙트럼분석, 칸다디아 균, 항칸다디아 활성

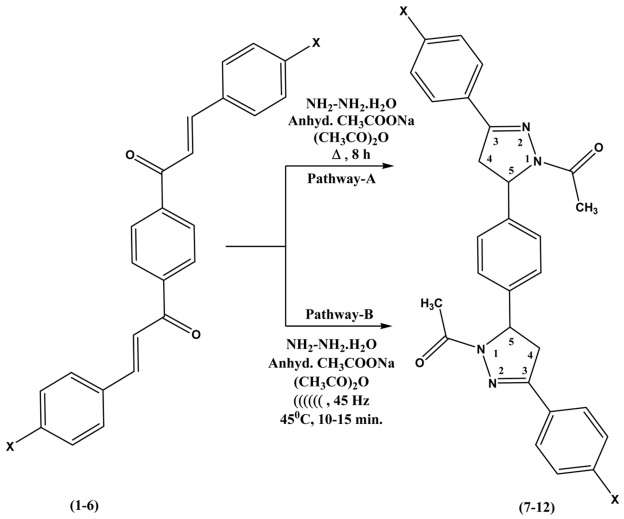
ABSTRACT. A new series of *bis* acetylated hybrid pyrazoles were synthesized and characterized by their melting point, elemental analysis, MS, FT-IR, one-dimensional ^{1}H, and ^{13}C NMR spectroscopic data. All the synthesized compounds were tested for their *in vitro* antifungal activities against *Candida sp.* namely *Candida albicans, Candida glabrata, Candida parapsilosis, Candida dubliniensis* and *Candida tropicalis.* A close inspection of the *in vitro* anticandidal activity profile in differently electron donating (CH_3 and OCH_3) and electron withdrawing (-F, -Cl, and Br) functional group substituted phenyl rings of novel hybrid pyrazoles exerted strong anticandidal activity against all the tested *Candida* species.
Keywords: Bis acetylated hybrid pyrazoles, *In situ* acetylation, Spectral analysis, *Candida sp*., Anticandidal activity

INTRODUCTION

An azole was a class of five-membered nitrogen heterocyclic ring compounds containing at least one heteroatom such as nitrogen, sulfur, or oxygen. Many azoles were used as antifungal drugs, inhibiting the fungal enzyme 14α-demethylase which produces ergosterol (an important component of the fungal plasma membrane). Some of the commercially available antifungal azoles were clotrimazole, posaconazole, ravuconazole, econazole, ketoconazole, voriconazole and fluconazole. Pyrazole derivatives with a phenyl group at the 5-position exhibited excellent characteristics of blue photoluminescence and electroluminescence.[1] Pyrazoles displayed various biological activities such as antimicrobial,[2] antifungal,[3] antidepressant,[4] immunosuppressive,[5] anticonvulsant,[6] anti-tumor,[7] antiamoebic,[8] antibacterial[9] and anti-inflammatory[10] activities. One sustainable strategy for green synthesis of organic compounds was ultrasonic irradiation. It accelerated the chemical reaction and mass transferred *via* the process of acoustic cavitation.[11] Compared to traditional methods, the procedure was more convenient to synthesis structurally diverse compounds[12] and could be carried out in higher yields in short reaction times under mild reaction conditions.

Candidiasis was an infection caused by a common type of fungus called Candida albicans and this fungus was found normally in the mouth, stomach, intestine, skin and vagina. It was easily controlled by our body immune system. The problem occured when it overgrows. Patients undergoing organ transplants, anticancer chemotherapy or long treatment with antimicrobial agents and patients with AIDS were immuno suppressed and very susceptible to life threatening systemic fungal infections like *Candidiasis*, *Cryptococcosis* and *Aspergillosis*. Antifungal azoles, fluconazole and itraconazole which were strong inhibitors of lanosterol 14α-demethylase (cytochrome *P*45014DM) and orally active have been widely used in

antifungal chemotherapy. Reports were available on the developments of resistance to currently available antifungal azoles in *Candida sp*., as well as clinical failures in the treatment of fungal infections.[13-15] Candidiasis was a fungal infection (mycosis) of any of the Candida species, of which Candida albicans was the most common.[16] Candidiasis encompassed infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. In continuation of our interest in synthesizing structurally diverse biologically active heterocycles,[17-20] we report now the '*one-pot*' synthesis of 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-aryl-pyrazol-5-yl)phenyl-3-arylpyrazoles, a novel series of *bis* acetylated hybrid pyrazole derivatives.



***Scheme* 1.** Synthesis of novel *bis* acetylated hybrid pyrazoles.

RESULTS AND DISCUSSION

Chemistry

1-Acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-aryl-pyrazol-5-yl)phenyl-3-arylpyrazoles **7-12** were synthesized in excellent yields by the reaction of *bis* chalcones **1-6** with hydrazine hydrate catalyzed by anhydrous sodium acetate/acetic anhydride under ultrasonic irradiation method at 45 °C within 10-20 min. Cavitation was (or might) responsible for acceleration of chemical reactions by ultrasound irradiation.[21] It has been observed in the traditional classical method, the reaction mixture of *bis* chalcones **1-6** with hydrazine hydrate catalyzed by anhydrous sodium acetate in refluxing acetic anhydride for 5-8 h yield compounds **7-12** in moderate yields. However when this reaction was performed under sonication method, the reaction took place rapidly within 10-20 min. with excellent yields (*Table* 1). In our present study, acetic anhydride was the best solvent for the facile synthesis of *bis* pyrazoles, **7-12** in excellent yields with out any solubility problem. In addition, *in situ* acetylation occured in the course of the reaction due to solvent, acetic anhydride under the reaction conditions. The structures of the synthesized 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-aryl-pyrazol-5-yl)phenyl-3-aryl pyrazoles **7-12** were confirmed by FT-IR, MS, ^{1}H NMR and ^{13}C NMR spectral studies and elemental analysis.

The formation of **7-12** could be rationalized on the basis of two reaction pathways. The first route involved the initial formation of a hydrazone followed by a subsequent 5-endo trig. ring cyclization, which according to Baldwin's rules was an unfavourable reaction. The second reaction pathway involved a Michael addition of hydrazine on the *bis* chalcones **1-6**, followed by a 5-exo-trig. ring cyclization and dehydration. This was an allowed process according to Baldwin's rules.[22] However, due to the tautomerism of pyrazolines, the products obtained by either of the mechanisms was the same 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-aryl-pyrazol-5-yl)phenyl-3-arylpyrazoles **7-12**.

Bioactive 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-phenyl-pyrazol-5-yl)phenyl-3-phenylpyrazole **7** was taken as the representative compound to elucidate the structure of the synthesized compounds. FT-IR spectrum

***Table* 1.** Physical and analytical data of *bis* acetylated hybrid pyrazoles **7-12**

Compounds	X	Time Δ (h) / sonication (min)	Yield (%) Δ / sonication	m.p. (°C)	Elemental analysis (%)			m/z $(M)^{+\cdot}$ Molecular formula
					C Found (calculated)	H Found (calculated)	N Found (calculated)	
7	H	7/15	65/95	261	74.55 (74.65)	5.69 (5.82)	12.31 (12.44)	450 $C_{28}H_{26}N_4O_2$
8	CH_3	5/10	65/98	258	75.13 (75.29)	6.22 (6.32)	11.60 (11.71)	478 $C_{30}H_{30}N_4O_2$
9	F	7/15	70/94	233	69.02 (69.12)	4.77 (4.97)	11.41 (11.52)	486 $C_{28}H_{24}F_2N_4O_2$
10	OCH_3	5/10	65/95	202	70.43 (70.57)	5.86 (5.92)	10.85 (10.97)	510 $C_{30}H_{30}N_4O_4$
11	Cl	8/20	55/88	260	64.52 (64.74)	4.52 (4.66)	10.66 (10.79)	518 $C_{28}H_{24}Cl_2N_4O_2$
12	Br	7/15	60/95	262	55.13 (55.28)	3.82 (3.98)	9.11 (9.21)	606 $C_{28}H_{24}Br_2N_4O_2$

of 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-phenyl-pyrazol-5-yl)phenyl-3-phenylpyrazole **7** showed characteristic absorption frequencies around 3057-3030 cm^{-1} due to aromatic CH stretching vibration. The absorption bands at 2923 and 2852 cm^{-1} were attributed to the aliphatic CH stretching vibration. The absorption frequency at 1659 cm-1 was assigned to amide carbonyl stretching vibration. The absorption band around 1441 and 1419 cm^{-1} were assigned to C=N stretching vibration. The absence of carbonyl band clearly supported the formation of 7, besides the disappearance of NH stretching vibration, which confirmed the *in situ* acetylation reaction due to acetic anhydride solvent. Mass spectrum of compound **7** showed molecular ion peak at m/z 450 ($M^{+\bullet}$), which was consistent with the proposed molecular formula of **7**. Elemental analysis of **7** (C_{cal} 74.65, C_{obs} 74.55; H_{cal} 5.82, H_{obs} 5.69; N_{cal} 12.44, N_{obs} 12.31) were consistent with the proposed molecular formula ($C_{28}H_{26}N_4O_2$) of **7**. In the ^{1}H NMR spectrum of **7**, the methylene protons (H-4a & H-4e) of the pyrazole moiety appeared as two doublets of doublet due to multiple coupling involving both geminal and vicinal protons. The signals for H-4a & H-4e were observed at 3.06 and 3.61 ppm. The doublet of doublet at 3.06 ppm ($J_{4a,5a}$=17.6, $J_{4a,4e}$=4.4 Hz) was assigned to H-4a proton of the pyrazoline moiety. Likewise, the doublet of doublet at 3.61 ppm ($J_{4e,4a}$=17.6 Hz & $J_{4e,5a}$=12.0 Hz) was assigned to H-4e proton of the pyrazole moiety. Similarly, the methine proton (H-5) of the pyrazoline moiety was expected to give signal as a doublet of doublet due to vicinal coupling with the two magnetically nonequivalent protons of the methylene group (H-4a & H-4e) of the pyrazoline moiety and the signals were observed at 5.48 ppm ($J_{5a,4a}$=11.8 Hz & $J_{5a,4e}$=4.6 Hz). Also, the acetyl methyl protons of pyrazoline moiety gave signal as a singlet at 2.31 ppm. The aromatic protons appeared as a multiplet in the range of 7.09-7.64 ppm. In the ^{13}C NMR spectrum of 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-phenyl-pyrazol-5-yl)phenyl-3-phenyl pyrazoles, the ^{13}C resonance at 59.60 ppm was assigned to C-5 of the pyrazole moiety. The ^{13}C resonance observed at 42.17 ppm was due to C-4 carbon of the pyrazole moiety. The ^{13}C resonance observed at 154.05 ppm was assigned to C-3 carbon of the pyrazole moiety. The aromatic carbons were observed in the region of 126.15-128.74 ppm. The remaining ^{13}C signals at 141.12, 131.28 and 130.36 ppm were due to *ipso* carbons. Therefore, with reference to FT-IR, MS, ^{1}H NMR and ^{13}C NMR spectral studies in compound **7**, the tentative assignments made for the title compounds were confirmed.

Anticandidal activity

The *in vitro* anticandidal activity of novel *bis* acetylated hybrid pyrazoles **7-12** was studied against the *Candida* species *viz., C.albicans, C.glabrata, C.parapsilosis, C.dubliniensis* and *C.tropicalis.* Fluconazole was used as a standard drug. Minimum inhibitory concentration (MIC) in μg/mL values was reproduced in *Table* 2 and their pictorial representation was shown in *Fig.* 1. A close survey of the MIC values indicated that all the tested bis acetylated hybrid pyrazole derivatives **7-12** exhibited a varied range (6.25-200 μg/mL) of anticandidal activity against all the tested *Candida* species except compounds **7** and **10**

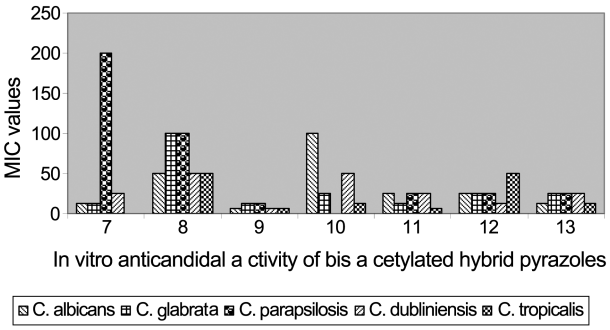


***Fig.* 1.** Pictorial representation of *in vitro* anticandidal activity (MIC) values for *bis* acetylated hybrid pyrazoles **7-12** (Compound **13** represents, standard drug Fluconazole).

***Table* 2.** *In vitro* anticandidal activity (MIC) values for *bis* acetylated hybrid pyrazoles **7-12**

Compounds	X	Minimum Inhibitory Concentration (MIC) in μg/mL				
		C. albicans	*C. glabrata*	*C. parapsilosis*	*C. dubliniensis*	*C. tropicalis*
7	H	12.5	12.5	200	25	-[a]
8	CH_3	50	100	100	50	50
9	F	6.25	12.5	12.5	6.25	6.25
10	OCH_3	100	25	-[a]	50	12.5
11	Cl	25	12.5	25	25	6.25
12	Br	25	25	25	12.5	50
Fluconazole		12.5	25	25	25	12.5

[a]-No inhibition even at higher concentration i.e., at 200 μg/mL

which were not having activity against *C.tropicalis* and *C.parapsilosis* respectively even at a higher concentration of 200 μg/mL. Compound **7**, having no substitution at the phenyl rings exerted moderate activity against all the tested *Candida* species and show MIC value in the range of 12.5-200 μg/mL. Two fold increase in activity was attained by **7** against *C.glabrata* when compared to standard drug Fluconazole and showed activity at a MIC value of 12.5 μg/mL, whereas **7** shows activity at a MIC value of 12.5 μg/mL against *C.albicans*. Compound **8** which has p-methyl substitution at the phenyl rings showed moderate activity against all the tested Candida sp., and show MIC value in the range of 50-100 μg/mL. Introduction of electron withdrawing fluoro functional group at the phenyl rings in compound **9** exerted excellent activity against all the tested *Candida* species which all show MIC in the range of 6.25-12.5 μg/mL. Replacement of electron withdrawing fluoro functional group at the phenyl rings in compound **9** by electron donating methoxy groups in compounds **10** exerted moderate anticandidal activity against all the tested *Candida* species in the MIC value range of 25-100 μg/mL except against *C.tropicalis* which showed activity at a MIC value of 12.5 μg/mL. Chloro substituted compound **11** exhibited excellent activity against *C.tropicalis* at a MIC value of 6.25 μg/mL whereas it showed MIC value of 12.5 μg/mL against *C.glabrata*. Compound **12**, which has bulky bromo substitution at the phenyl rings exhibited good activity against all the tested strains except against *C.tropicalis* which showed MIC value of 12.5 μg/mL against *C.dubliniensis*.

EXPERIMENTAL

General

Sonication was performed on a Life Care - Fast Ultrasonic system operating at a frequency of 45 kHz. The reaction flask was located in the maximum energy area in the bath and the addition or removal of water controlled the temperature of the water bath. IR spectra were recorded in KBr (pellet forms) on a Thermo Nicolet-Avatar-330 FT-IR spectrophotometer and note worthy absorption values (cm^{-1}) alone were listed. ^1H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively on Bruker AMX 400 NMR spectrometer using $CDCl_3$ as solvent. The ESI +ve MS spectra were recorded on a Varian Saturn 2200 MS spectrometer. Satisfactory microanalyses were obtained on Carlo Erba 1106 CHN analyzer. Performing TLC assessed the reactions and the purity of the products. All the reported melting points were taken in open capillaries and were uncorrected. By adopting the literature precedent, *bis* chalcones **1-6**[23] were prepared.

Experimental procedure for the synthesis of novel 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-arylpyrazol-5-yl)phenyl-3-arylpyrazoles 7-12 under classical thermal method

Bis chalcones **1-6**, (0.01 mol), hydrazine hydrate (0.01 mol), anhydrous sodium acetate (0.01 mol) and acetic anhydride (20 mL) were taken in a round bottomed flask and the reaction mixture flask was refluxed until the products were formed. The reaction was monitored by TLC. The time required for the formation of various pyrazoles was shown in *Table* 1. The reaction mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried and the obtained solids were purified by column chromatography using toluene and ethylacetate (1:1) mixture as eluent which afforded the title compounds **7-12** in moderate yields.

Experimental procedure for the synthesis of novel 1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-arylpyrazol-5-yl)phenyl-3-arylpyrazoles 7-12 under ultrasound irradiation

Bis chalcones **1-6**, (0.01 mol), hydrazine hydrate (0.01 mol), anhydrous sodium acetate (0.01 mol) and acetic anhydride (20 mL) were taken in a conical flask and the reaction mixture flask was suspended at the centre of the ultrasonic bath to get the maximum ultrasound energy and sonicated until the products were formed. The reaction was monitored by TLC. The time required for the formation of various pyrazoles was shown in *Table* 1. The reaction mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried and recrystallized from acetic acid to afford pale yellow coloured crystals.

Spectral data

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-phenyl-pyrazol-5-yl)phenyl-3-phenylpyrazole 7: IR (KBr) (cm^{-1}): 3057, 3030, 2923, 2852, 1659, 1441, 1419, 763, 690, 559; ^1H NMR (δ ppm): 2.31 (s, 3H, acetyl CH_3), 3.06 (dd, 2H, H_{4a}, $J_{4a,5a}$=17.6, $J_{4a,4e}$=4.4 Hz), 3.61 (dd, 2H, H_{4e}, $J_{4e,4a}$=17.6, $J_{4e,5a}$=12.0 Hz), 5.48 (dd, 2H, H_{5a}, $J_{5a,4a}$=11.8, $J_{5a,4e}$=4.6 Hz), 7.09-7.64 (m, 14H, $H_{arom.}$); ^{13}C NMR (δ ppm): 21.94 Acetyl CH_3, 154.05 C-3, 42.17 C-4, 59.60 C-5, 168.88 Amide C=O, 126.15-128.74 –$C_{arom.}$, 141.12, 131.28, 130.36 *ipso* carbons.

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-(4-methylphenyl)-pyrazol-5-yl)phenyl-3-(4-methylphenyl)pyrazole 8: IR (KBr) (cm^{-1}): 3063, 3035, 2958, 2923, 2853, 1659, 1446, 1421, 633, 585, 543; ^{1}H NMR (δ ppm): 2.30 (s, 3H, acetyl CH_3), 2.42 (s, 6H, CH_3 at phenyl rings), 3.14 (dd, 2H, H_{4a}, $J_{4a,5a}$=17.1, $J_{4a,4e}$=4.4 Hz), 3.68 (dd, 2H, H_{4e}, $J_{4e,4a}$=17.6, $J_{4e,5a}$=12.0 Hz), 5.56 (dd, 2H, H_{5a}, $J_{5a,4a}$= 11.8, $J_{5a,4e}$=4.6 Hz), 7.17-7.63 (m, 12H, $H_{arom.}$); ^{13}C NMR (δ ppm): 21.52 CH_3 at phenyl rings, 21.94 Acetyl CH_3, 154.14 C-3, 42.22 C-4, 59.51 C-5, 168.77 Amide C=O, 126.13-129.44 –$C_{arom.}$, 141.15, 140.68 *ipso* carbons.

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-(4-fluorophenyl)-pyrazol-5-yl) phenyl-3-(4-fluorophenyl)pyrazole 9: IR (KBr) (cm^{-1}): 3046, 2958, 2923, 2852, 1655, 1446, 1417, 632, 579, 544; ^{1}H NMR (δ ppm): 2.30 (s, 3H, acetyl CH_3), 3.04 (dd, 2H, H_{4a}, $J_{4a,5a}$=13.6, $J_{4a,4e}$= 4.2 Hz), 3.60 (dd, 2H, H_{4e}, $J_{4e,4a}$=17.6, $J_{4e,5a}$=12.0 Hz), 5.48 (dd, 2H, H_{5a}, $J_{5a,4a}$=11.8, $J_{5a,4e}$=4.6 Hz), 7.00-7.64 (m, 12H, $H_{arom.}$); ^{13}C NMR (δ ppm): 21.91 Acetyl CH_3, 152.99 C-3, 42.23 C-4, 59.68 C-5, 168.82 Amide C=O, 115.80-128.62 –$C_{arom.}$, 141.08, 141.04 *ipso* carbons.

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-(4-methoxyphenyl)-pyrazol-5-yl)phenyl-3-(4-methoxyphenyl)pyrazole 10: IR (KBr) (cm^{-1}): 3063, 3008, 2923, 2852, 1657, 1453, 1430, 561, 580, 549; ^{1}H NMR (δ ppm): 2.27 (s, 3H, acetyl CH_3), 3.08 (dd, 2H, H_{4a}, $J_{4a,5a}$=17.5, $J_{4a,4e}$=5.0 Hz), 3.80 (signal merged with OCH_3 protons), 3.80 (s, 6H, OCH_3 at phenyl rings), 5.49 (dd, 2H, H_{5a}, $J_{5a,4a}$=11.5, $J_{5a,4e}$=4.5 Hz), 6.99-7.72 (m, 12H, $H_{arom.}$); ^{13}C NMR (δ ppm): 22.11 Acetyl CH_3, 154.40 C-3, 42.56 C-4, 55.82 OCH_3 at phenyl rings, 59.43 C-5, 167.57 Amide C=O, 114-68-128.77 –$C_{arom.}$, 141.86, 161.41 *ipso* carbons.

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-(4-chlorophenyl)-pyrazol-5-yl)phenyl-3-(4-chlorophenyl) pyrazoles 11: IR (KBr) (cm^{-1}): 3041, 2958, 2923, 2852, 1655, 1441, 1420, 637, 565, 543; ^{1}H NMR (δ ppm): 2.38 (s, 3H, acetyl CH_3), 3.12 (dd, 2H, H_{4a}, $J_{4a,5a}$=17.6, $J_{4a,4e}$= 4.1 Hz), 3.68 (dd, 2H, H_{4e}, $J_{4e,4a}$=17.2, $J_{4e,5a}$=12.0 Hz), 5.58 (dd, 2H, H_{5a}, $J_{5a,4a}$=11.2, $J_{5a,4e}$=4.8 Hz), 7.17-7.65 (m, 12H, $H_{arom.}$); ^{13}C NMR (δ ppm): 21.95 Acetyl CH_3, 152.92 C-3, 42.06 C-4, 59.76 C-5, 168.89 Amide C=O, 126.13-129.78 –$C_{arom.}$, 136.31, 141.01, 141.05 *ipso* carbons.

1-acetyl-4,5-dihydro-5(4-(1-acetyl-4,5-dihydro-3-(4-bromophenyl)-pyrazol-5-yl)phenyl-3-(4-bromophenyl) pyrazole 12: IR (KBr) (cm^{-1}): 3035, 2958, 2922, 2852, 1652, 1441, 1420, 671, 561, 552; ^{1}H NMR (δ ppm): 2.38 (s, 3H, acetyl CH_3), 3.12 (dd, 2H, H_{4a}, $J_{4a,5a}$=13.4, $J_{4a,4e}$ =4.2 Hz), 3.68 (dd, 2H, H_{4e}, $J_{4e,4a}$=17.4, $J_{4e,5a}$=11.8 Hz), 5.57 (dd, 2H, H_{5a}, $J_{5a,4a}$=11.8, $J_{5a,4e}$=5.0 Hz), 7.16-7.57 (m, 12H, $H_{arom.}$); ^{13}C NMR (δ ppm): 21.95 Acetyl CH_3, 152.96 C-3, 42.01 C-4, 59.76 C-5, 168.89 Amide C=O, 124.67-128.02 –$C_{arom.}$, 130.22, 131.97, 141.01 *ipso* carbons.

Microbiology

Materials: All the clinically isolated fungal strains namely *Candida albicans, Candida glabrata, Candida parapsilosis*, *Candida dubliniensis* and *Candida tropicalis* were obtained from Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

***In vitro* anticandidiasis activity:** Minimum inhibitory concentration (MIC) in μg/mL values was carried out by two-fold serial dilution method.[24] The respective test compounds **7-12** were dissolved in dimethyl sulphoxide (DMSO) to obtain 1 mg mL^{-1} stock solution. Seeded broth (broth containing microbial fungal spores) was prepared at 37 ± 1 ºC from 1 to 7 days old Sabourauds agar (Himedia, Mumbai) slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4-10^5 cfu/mL. The final inoculums size was 1.1-1.5 X 10^2 cfu/mL for antifungal assay. Testing was performed at a pH 5.6 for fungi (SDB). Exactly 0.4 mL of the solution of test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 28 ± 1 ºC for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 72-96 h (for fungi) of incubation. Fluconazole was used as standard drug for *Candida species*.

CONCLUSION

In crunch, a series of novel bis acetylated hybrid pyrazoles **7-12 we**re synthesized and characterized by their spectroscopic data. Compound **7** against *C.albicans* and *C.glabrata,* Compound **9** against C.glabrata and C.parapsilosis, compound **11** against C.glabrata, Compound **12** against *C.dubliniensis* exerted admirable anticandidal activity at a MIC value of 12.5 μg/mL. Compound **9** against *C.albicans*, *C.dubliniensis*, *C.tropicalis*, Compound **11** against C.tropicalis exhibited excellent activity at a MIC value of 6.25 μg/mL. Results of the biological activity show that electron withdrawing substitutents like fluoro, chloro and bromo substituted derivatives exerted excellent antibacterial and antifungal activities, since electron

withdrawing substituent increased the lipophilicity due to the strong electron withdrawing capability.[25] Moreover, electron withdrawing substitutents namely fluorine substitution was commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein ligand interactions.[26] These observations may promote a further development of our research in this field. Furthermore, the observed marked anticandidiasis activity of this group of *bis* acetylated hybrid pyrazole derivatives may be considered as key steps for the building of novel chemical entities with comparable pharmacological profiles to that of the potent standard drugs.

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