Journal of Young Pharmacists, 2023; 15(4):638-648.
https://www.jyoungpharm.org

Original Article

Synthetic Identification of New Compounds with Anti-fungal Properties of "1-[3-(2-Hydroxyphenyl)-3-Oxop ropanoyl]- 5,5-Diphenylimidazolidine -2,4-Dione and its Derivatives"

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ABSTRACT

Background: 5, 5-diphenylimidazolidine is a heterocyclic hydrocarbon having unique basic structural characteristics in their molecular structure. In this research; we synthesis various 11 derivatives of -[3-(2-hydroxyphenyl)-3-oxopropanoyl]- 5,5-diphenylimidazolidine -2,4-dione with anti-fungal activity. The agar well diffusion method was used to conduct the pharmacological screening for antifungal activity. **Materials and Methods:** All 5, 5-diphenylimidazolidine derivatives were synthesized by conventional method. Benzoin; Benzil; Urea; Glacial Acetic Acid; 4- hydroxyl benzoic acid; Con. HNO_3; Formic Acid; 2- Nitro Aniline are used for the synthesis. The structure confirmations were done by FTIR, NMR spectroscopy and MS. **Results:** In this research; we concluded that; many derivatives give potent anti-fungal activity against fungi. The compound 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ); 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BG); 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BE); shown better antifungal activity against *Candida albicans*. BJ was shown to be the most effective chemical when compared to Griseofulvin and other common medications because it demonstrated greater antifungal action against *Aspergillus niger*. **Conclusion:** The anti-fungal activity of the title compounds and their derivatives was studied. Studies on the link between structure and activity revealed that compounds containing 5, 5-dimethylimidazolidinone derivatives with an electron-withdrawing group have higher activity than those containing an electron-donating group. 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BE); 1-[3-(oxo(phenyl-amino) acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BK) shown better antifungal activity against *Candida albicans*.

Keywords: 5, 5-diphenylimidazolidine, Benzil, Urea, 4- amino benzoic acid, 2-Nitro Aniline, 4-Nitro Aniline, Aniline, Anti-fungal Activity, Griseofulvin.

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Received: 07-08-2023;
Revised: 20-08-2023;
Accepted: 02-09-2023.

INTRODUCTION

In 1954, the 5, 5-diphenylimidazolidine nucleus was found. It has an amalgamated di-benzene and imidazolidine ring. Its structure resembles that of the medication phenytoin.[1] Due to its numerous pharmaceutical applications, 5, 5-diphenylimidazolidine has significant heterocyclic nuclei. Scientist Brecker created the first 5, 5-diphenylimidazolidine in 1956.[2] Two nitrogen atoms are present in 5, 5-diphenylimidazolidine-2, 4-dione (Figure 1).

The molecular weight of 5, 5-diphenylimidazolidine -2, 4-dione is 252.273 and the molecular formula of 5, 5-diphenylimidazolidine-2,4-dione is $C_{15}H_{14}N_2O_2$.[3] In particular, a molecule with an attached 5, 5-diphenylimidazolidine-2,4-dione ring that had both electron withdrawing and electron donating groups shown greater inhibitory ability than normal medication against both bacterial and fungal strains. A wide range of activities, including antimicrobial, anti-inflammatory, analgesic, anti-tubercular, anti-hypertensive, anti-convulsant, and anti-viral activity, are provided by 5,5-diphenylimidazolidine-2,4-dione.[4] Human mucosal health is somewhat impacted by microbial fungi like *Candida albicans* and *Aspergillus niger*. These microbial growths cause the host tissue to be destroyed and can cause fatal infections. Fungal infections of the skin and nails are brought on by the parasites *Candida albicans* and *Aspergillus niger*.

DOI: 10.5530/jyp.2023.15.89

Millions of people in poorer nations are also impacted. Up to 1, 30,000 people per year die from these illnesses worldwide, infecting more than 60 million people.[5] Although they are the most frequently prescribed medications for this bacterial illness, they have serious side effects. Therefore, great efforts have been undertaken by young scientists around the world and by numerous research groups to discover novel antibacterial drugs. The 5,5-diphenylimidazolidine-2,4-dione and its derivatives, on the other hand, are one of the most significant groups of organic heterocyclic compounds having anti-microbial activity, including anti-bacterial, anti-fungal, herbicidal, and anti-viral activities.[6-8] Based on these contributions, we will continue our drug research programme involving the creation of new, safer, and more biologically active derivatives,[9,10] in the hopes that this will spark interest in the creation of a new series of 5,5-diphenylimidazolidine-2,4-dione that will result in more potent and less harmful antifungal agents.

MATERIALS AND METHODS

Materials

Benzoin; Benzil; Urea; Glacial Acetic Acid; 4- hydroxy benzoic acid; Con. HNO_3; Formic Acid; 2- Nitro Aniline; 4- Nitro Aniline; Aniline; Acetyl Chloride; Formic Acid; 4- amino Phenol; 3- Nitro Aniline etc. are used for the synthesis. Analytical grade chemicals were used throughout. Some chemicals are available at colleges; however, all were obtained from Modern Chemicals in Nashik.

Methods

By using a traditional approach, all five 5-diphenylimidazolidine derivatives were created. By using the open tube capillary method, melting points were measured and determined. The chemicals' purity was examined using Thin Layer Chromatography (TLC) techniques. IR spectra were collected using KBr pellets and a Perkin Elmer Spectrum FTIR spectrometer. TMS was used as the internal standard in DMSO-d6/CDCl3 to record 1H-NMR spectra on a Bruker AVANCE III 500 MHz (AV 500) spectrometer. Two mass spectra were collected on a JEOL GCMATE II MS and are shown as m/z. The process by which derivatives of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BD) are depicted in Schemes 1A and 1B.

Experimental Work

Chemistry: (Scheme IA)

(Scheme IB)

Procedure

Synthesis of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione (BD) Derivatives:

Synthesis of Benzil (BA)- (scheme 1A)

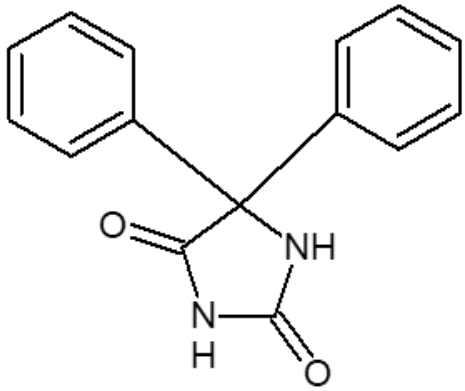
4 g of benzoin and 14 mL of concentrated nitric acid are heated in a round bottom flask for 1.5 hr on a hot water bath. Once the reaction is complete, add 75 mL of water, cool the mixture to room temperature, and stir for a few minutes to coagulate the precipitate. Gather the goods using a Buchner funnel. After thoroughly pressing the product to remove moisture, 10 cc of ethanol was used to re-crystallize the substance in question. After the substance has been dissolved, add water drop by drop until the cloud point is reached. Re-crystallize the product if you can. Gather the result on a Buchner funnel when it has recrystallized, then dry it.

Synthesis of 5,5-diphenylimidazolidine -2,4-dione (BB)-(scheme 1A)

Take 5.3 g of Benzyl in 100 mL RBF they add 3.0 g of urea in that RBF Then add 15 mL 30% aq. NaOH (sodium hydroxide) Lastly add 75 mL of C_2H_5OH (ethanol) Attach reflux condenser and boil under reflux using heating mantle for at least 2 hr. To a comfortable temperature Mix thoroughly after adding the reaction mixture product to 125 mL of water. Allow to stand for 10 min Then filter under suction pump to remove an insoluble by-product. Render the product filtrate with strongly acidic acid with concentrated HCl Cool in Ice water and immediately filter off ppt.

Synthesis of 1-acetyl-5,5-diphenylimidazolidine -2,4-dione(BC)-(scheme 1A)

Take 1 g of 5,5-diphenylimidazolidine -2,4-dione, 1 mL of glacial acetic acid and 25 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture at about 80-100°C for 2 hr (Refluxing the reaction mixture) Cool the reaction mixture by adding 10 mL of crushed ice. Again, add ice cold water. Filter the reaction mixture and collect the product. Record Melting point, Theoretical yield, Practical yield and % of Practical yield.



5,5-diphenylimidazolidine-2,4-dione

Figure 1: 5, 5-diphenylimidazolidine heterocyclic nucleus.

Synthesis of 1-[3-(2-hydroxyphenyl)-3-oxo propanoyl]- 5,5-diphenylimidazolidine -2,4-dione(BD)-(scheme 1A)

Take 1 g of 1-Acetyl-5,5-Diphenylimidazolidine-2,4-Dione, 1 g of 2-hydroxy benzoic acid and 25 mL of ethanol in a flask with a circular bottom. Reflux the reaction mixture at about 80-100°C for 2 and ½ hr. Cool the reaction mixture by adding 5 mL of crushed ice water. Filter the reaction mixture and collect the product. Record Melting point, Theoretical yield, Practical yield and % of Practical yield.

Synthesis of 1-[3-(*N*-Phenyl-2-nitroaniline)-3-ox opropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BE)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 g of 2-nitro aniline and 25 mL of ethanol in a flask with a circular bottom. The reaction mixture is heated under reflux conditions for 1 hr. After completion of the reaction remove the RBF from the hot water bath and allow to cool the reaction mixture then add 50 mL of ice-cold water into the reaction mixture then allow to stand it for 5 min. filter the product and recrystallized with ethanol to give 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxo propanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesis of 1-[3-(*N*-Phenyl-4-nitroaniline)-3-ox opropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BF)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 4-nitro aniline and 25 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture for 1 hr. after the completion of reaction allow to cool the reaction mixture at room temperature. add 50 mL ice cold water into the mixture. product was filtered and recrystalized from ethanol to give 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxo propanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesis of 1-[3-(*N*-phenylformamide)-3-ox opropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BG)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 5 mL of formic acid then add 30 mL of ethanol in a flask with a circular bottom. Reflux the reaction mixture on hot water bath for 1 and ½ hr. after completion of reaction allow the reaction to cool, add ice cold water to the reaction mixture filter the reaction mixture and recrystalized it with ethanol to give 1-[3-(*N*-phenylformamide)-3-oxo propanoyl]-5,5-diphenylimidazolidine-2,4-dione as product.

Synthesis of 1-[3-(2-oxyphenyl)amino]benzoic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BH)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 g of benzoic acid then add 30 mL of ethanol in a flask with a circular bottom. Shake it well. Heat the reaction mixture for 1 hr. After 1 hr cool the reaction mixture at room temperature and add 50 mL of ice-cold water. Allow to stand it for 5 min filter the product and dry and recrystalized with ethanol to give the 1-[3-(2-oxyphenyl)amino]benzoic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione as product.

Synthesis of 1-[3-(2-aminophenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BI)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 mL of aniline then add 30 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture for 1 hr. After completion of reaction allow the reaction mixture to cool by adding ice cold water. filter and dry the product and recrystalized with ethanol to give 1-[3-(2-aminophenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesis of 1-[3-(2-phenyl acetate)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BJ)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione, 2 mL of acetyl chloride then add 30 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture at reflux condition at constant 50°C for 3 hr as the acetyl chloride is explosive and flammable (heat the reaction mixture at the lowest temperature). after completion of reaction put the RBF for few min aside add ice cold water to the mixture. Filter and dry the product and recrystalized with ethanol to give 1-[3-(2-phenyl acetate)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione as product.

Synthesis of 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BK)-(scheme-1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 mL of acetic acid then add 30 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture for 1 hr after completion of reaction add ice cold water to the mixture. Filter and dry the product and recrystalized with ethanol to give 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesis of 1-[3-(*N*-phenyl benzene)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BL)-(scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 g of N-Phenyl Benzene then add 30 mL of ethanol in a flask with a circular bottom. Heat the mixture for 1 and ½ hr after completion of reaction allow the reaction mixture to cool by adding ice cold water to the mixture. Filter and dry the product. Recrystalized it with ethanol to give 1-[3-(*N*-phenyl benzene)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesis of 2-(2-(3-(2,4-dioxo-5,5-diphenylimidazolidin-1-yl)-3-oxopropanoyl) phenoxy)-2-oxoacetic acid (BM)- (scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 g of oxalic acid then add 30 mL of ethanol in a flask with a circular bottom. Heat the reaction mixture for 1 hr after completion of reaction add ice cold water to the mixture filter and dry the product and recrystalized with ethanol to give 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.

Synthesisof1-(3-(2-(4-aminophenoxy) phenyl)-3-oxopropanoyl)-5,5-diphenylimidazolidine-2,4-dione (BN)- (scheme 1B)

Take 2 g of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine -2,4-dione, 2 g of 4-aminophenol then add 30 mL of ethanol in a flask with a circular bottom. Heat the mixture for 1 and ½ hr after completion of reaction allow the reaction mixture to cool by adding ice cold water to the mixture. Filter and dry the product. Recrystalized it with ethanol to give 1-[3-(N-phenyl benzene)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.

RESULTS

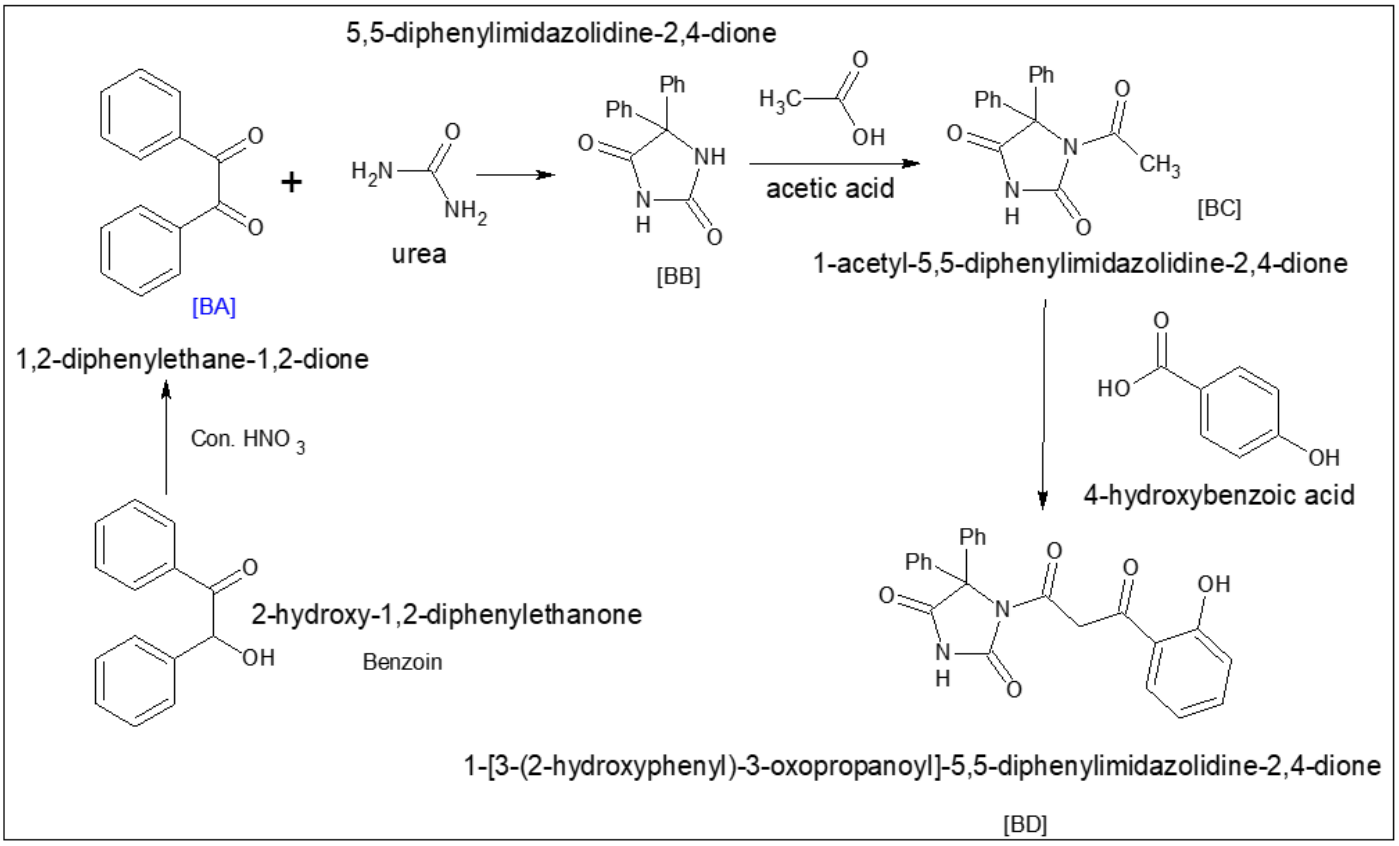
Characterization

The physical data of derivatives of 1-[3-(2-hydroxyphenyl)-3-oxo propanoyl]- 5,5-diphenylimidazolidine-2,4-dione (BD) were displayed in Table 1 .

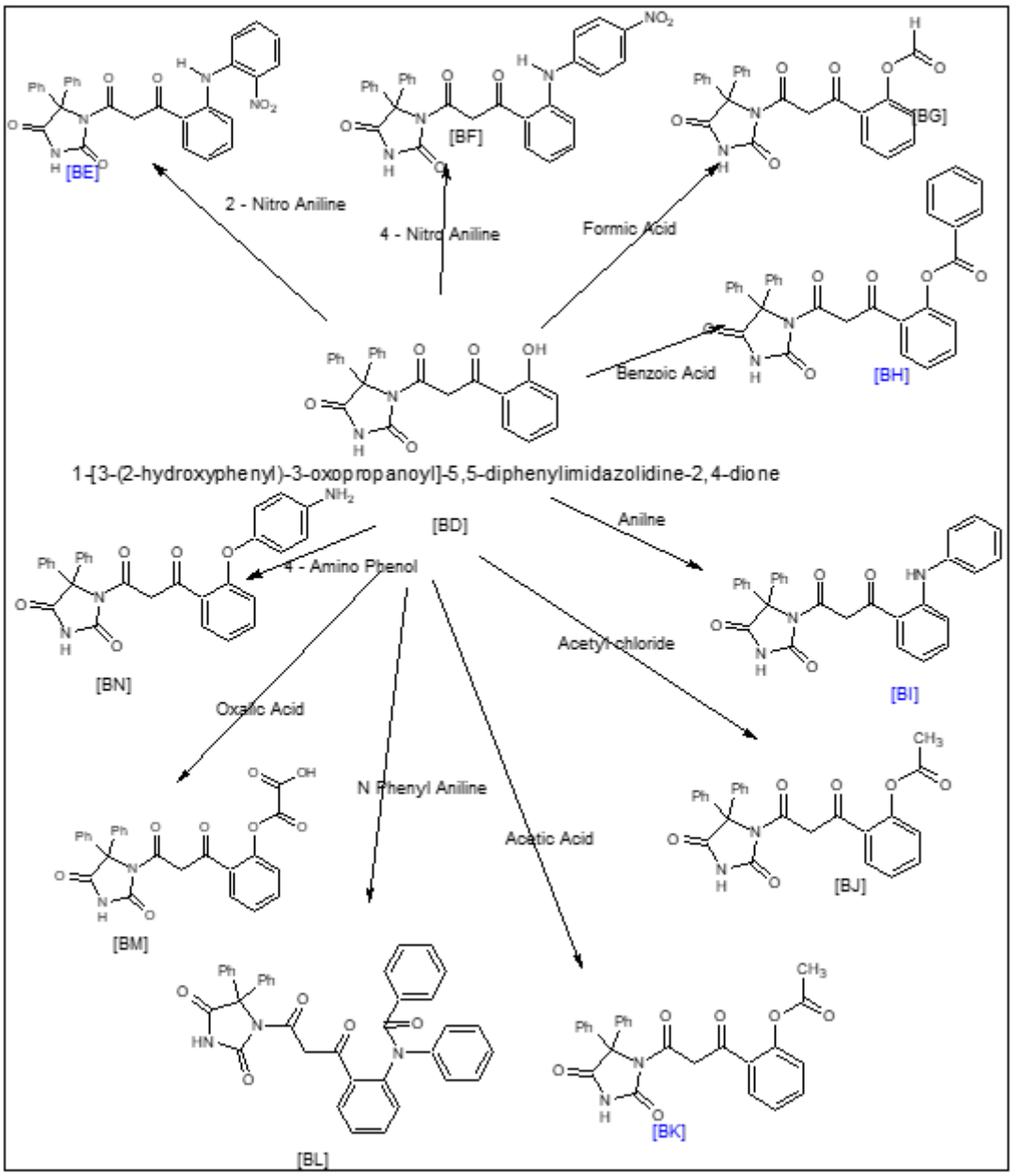
Spectral Data

Synthesis of 1-[3-(N-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BG)-(scheme 1B)

FTIR (KBr) ν cm^{-1}: 1674.7 C=C Stretch (Aromatic), 1138.90 C-C Stretch (Aromatic), 3250.6 C-N Stretch (Aromatic), 1662.74 N-H



Scheme 1A: Synthesis of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione.



Scheme 1B: Synthesis of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione) derivatives (BE- BK).

Stretch (Aromatic), 1686.6 C=O Stretch (Aryl ketone),1494.10 Amide NH-C=O Structure; ^{1}H NMR (400 MHz, DMSO): δ 12.172 Ar N-H (s, 1H), δ 12.088 N-H (s, 1H), δ 8.721-7.158 Ar C-H (m, 18H), δ 3.326 CH_2 Group (s, 2H); Mol.Wt: 534.

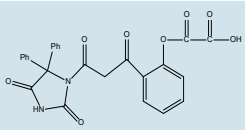
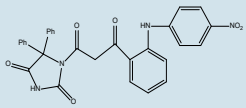
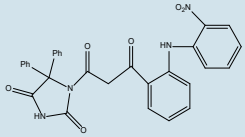
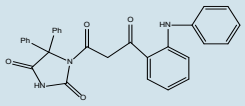
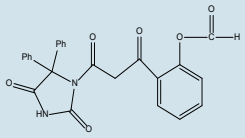
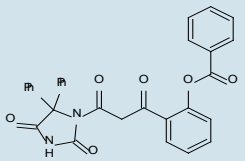
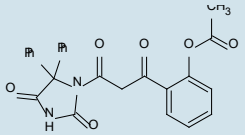
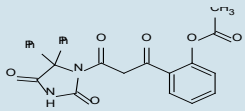
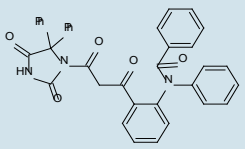
Synthesis of 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BE)-(scheme 1B)

FTIR (KBr) ν cm^{-1}: 1663.04 C=C Stretch (Aromatic), 1072.42 C-C Stretch (Aromatic), 1344.88 C-N Stretch (Aromatic), 3485 N-H Stretch (Aromatic), 1623.9 C=O Stretch (Aryl ketone), 1692.42 Amide NH-C=O, 1724.9 R-O-R' (Aromatic stretch); 1H NMR (400 MHz, DMSO): δ 11.506 Ar N-H (s, 1H), δ 10.624 N-H (s, 1H), δ 8.717-7.158 Ar C-H (m, 18H), δ 3.348 CH_2 Group (s, 2H); Mol.Wt: 534.

Synthesis of 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BK)-(scheme-1B)

FTIR (KBr) ν cm^{-1}: 1663.06 C=C Stretch (Aromatic),1077.16 C-C Stretch (Aromatic), 1266.75 C-N Stretch (Aromatic), 3225.92 N-H Stretch (Aromatic), 1673.73 C=O Stretch (Aryl ketone), 3398.4 N-H Aniline; 1H NMR (400 MHz, DMSO): δ 12.075 Ar N-H (s, 1H), δ 11.624 N-H (s, 1H), δ 8.471-6.949 Ar C-H (m, 19H), δ 3.348 CH_2 Group (s, 2H); Mol.Wt: 465.

Table 1: Physical Data of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]- 5,5-diphenylimidazolidine -2,4-dione (BD) Derivatives.

Code	Structure	Molecular formulae	Molecular weight (g)	Melting point (°C)	Yield (%)	Rf value
BM		$C_{26}H_{18}N_2O_8$	486.44	166-170	70.23	0.78
BF		$C_{30}H_{22}N_4O_6$	534.53	170-173	80.25	0.70
BE		$C_{30}H_{22}N_4O_6$	534.53	169-174	76.23	0.80
BI		$C_{30}H_{23}N_3O_4$	489.53	176-178	80.45	0.77
BG		$C_{25}H_{18}N_2O_6$	442.43	178-180	78.48	0.81
BH		$C_{25}H_{20}N_2O_4$	412.45	164-169	75.23	0.74
BJ		$C_{28}H_{24}N_2O_4$	452.51	172-176	82.49	0.75
BK		$C_{30}H_{23}N_3O_5$	505.53	168-172	80.42	0.81
BL		$C_{28}H_{22}N_2O_8$	514.49	167-171	85.28	0.83

Synthesis of 1-[3-(2-oxyphenyl)amino]benzoic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BH)-(scheme 1B)

FTIR (KBr) ν cm^{-1}: 1673.73 C=C Stretch (Aromatic), 1138.90 C-C Stretch (Aromatic),3238.7 N-H Stretch (Aromatic), 1662.74 C=O Tertiary Amide, 1488.18 R-O-R' (Aromatic stretch); 1H NMR (400 MHz, DMSO): δ 10.695 Ar N-H (s, 1H), δ 8.464-7.927Ar C-H (m, 14H), δ 3.676 CH_2 Group (s, 2H); Mol.Wt: 442.

Synthesis of 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BF)-(scheme 1B)

FTIR (KBr) ν cm^{-1}: 1673.76 C=C Stretch (Aromatic), 3250.6 N-H Stretch (Aromatic), 1662.74 C=O Stretch (Aryl ketone), 1670.6 C=O Tertiary Amide, 1488.40 R-O-R' (Aromatic stretch), 1686.6 C=O COOH, 1138.90 C-O, 924 O-H (Bend); 1H NMR (400 MHz, DMSO): δ 12.955 Ar N-H (s, 1H), δ 8.266-7.483 Ar C-H

Table 2: *In vitro* Anti-fungal activity [MIC's in (mg/mL)] of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]- 5,5-diphenylimidazolidine -2,4-dione (BD).

Compound	Code No.	Concentration (µmg)	Culture	
			Candida albicans	*Aspergillus niger*
BF	K1	25 mg/mL	8.8	9.9
	K2	50 mg/mL	12.5	13.4
	K3	100 mg/mL	19.8	21.2
BJ	K1	25 mg/mL	7.5	8.8
	K2	50 mg/mL	13.2	14.2
	K3	100 mg/mL	19.3	20.3
BE	K1	25 mg/mL	12.4	13.4
	K2	50 mg/mL	19.8	21.5
	K3	100 mg/mL	19.3	12.4
BG	K1	25 mg/mL	8.5	19.8
	K2	50 mg/mL	12.5	13.4
	K3	100 mg/mL	12.7	21.2
BK	K1	25 mg/mL	8.8	9.9
	K2	50 mg/mL	12.4	13.4
	K3	100 mg/mL	19.8	21.2
BM	K1	25 mg/mL	7.5	8.8
	K2	50 mg/mL	13.2	14.2
	K3	100 mg/mL	19.3	20.3
STD Griseofulvin	K1	25 mg/mL	8.5	7.5
	K2	50 mg/mL	12.5	11.4
	K3	100 mg/mL	24.5	25.2

Values are expressed in mean ± SD, (*n*=3).

(m, 14H), δ 3.334 CH_2 Group (s, 2H), 2.761 Ar CH_3 group (s, 3H); Mol.Wt: 428.

Synthesis of 1-[3-(2-phenyl acetate)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BJ)-(scheme 1B)

FTIR (KBr) ν cm^{-1}: 1686.6 C=C Stretch (Aromatic), 1138.90 C-C Stretch (Aromatic), 1286 C-N Stretch (Aromatic), 3398.4 N-H Stretch (Aromatic), 1600.15 C=O Stretch (Aryl ketone), 2868.4C_4H_7 (C-H); 1H NMR (400 MHz, DMSO): δ 13.088 Ar N-H (s, 1H), δ 7.893-7.236 Ar C-H (m, 17H), δ 3.589 CH_2 Group (s, 2H); Mol.Wt: 452.

Synthesis of 2-(2-(3-(2,4-dioxo-5,5-diphenylimidazolidin-1-yl)-3-oxopropanoyl) phenoxy)-2-oxoacetic acid (BM- Scheme 1)

FTIR (KBr) ν cm^{-1}:1686.6 C=C Stretch (Aromatic), 1138.90 C-C Stretch (Aromatic), 1319.60 C-N Stretch (Aromatic), 3398.4 N-H Stretch (Aromatic), 770.41 Disubstituted Aromatic Ring -, 2558.7 Dihydrate (COOH), 1724.60 C=O -, 1319.60 C-O, 948.57 O-H; 1H NMR (400 MHz, DMSO): δ 12.955 Ar N-H (s, 1H), δ 8.266-7.483 Ar C-H (m, 14H), δ 3.334 CH_2 Group (s, 2H), 2.761 Ar CH_3 group (s, 3H); Mol.Wt: 486.

Biological evaluation

In vitro Antimicrobial Activity by Agar Well Diffusion Method

The agar well diffusion method was used to assess substances' antifungal or antimicrobial properties. Mueller Hinton Agar (MHA) was prepared according to the manufacturer's instructions, and 100 L of fungal suspension containing approximately 1.5 108 CFU/mL was evenly dispersed onto the agar plates' surface. A sterile cork borer was used to create sterile 6-mm wells, and each well was filled with 50 L of the test substance at varied concentrations (25, 50, and 100 g/mL). The standard reference chemical was Griseofulvin. For bacteria, the plates were incubated at 37°C for 24 hr. The diameter of the inhibitory zone was measured in millimetres after incubation, and the results were recorded. Each test was repeated three times, and the mean value was computed. The agar dilution method was used to determine the Minimum Inhibitory Concentration (MIC). In 96-well microplates, the test chemicals were dissolved in DMSO and put to Mueller Hinton agar. Fungal suspensions were introduced to the wells after two-fold serial dilutions were made.[11,12] After that,

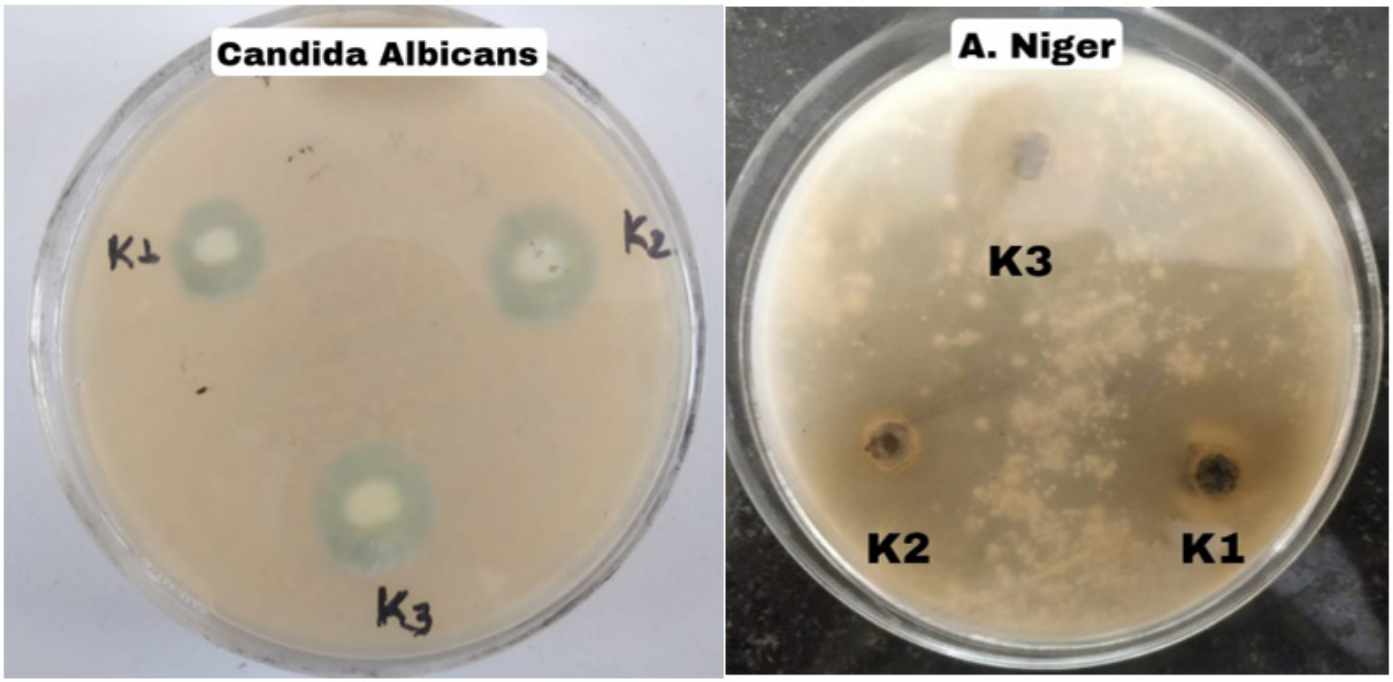


Figure 2: Zone of Inhibition of 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BF) (*Candida albicans and Aspergillus niger*).

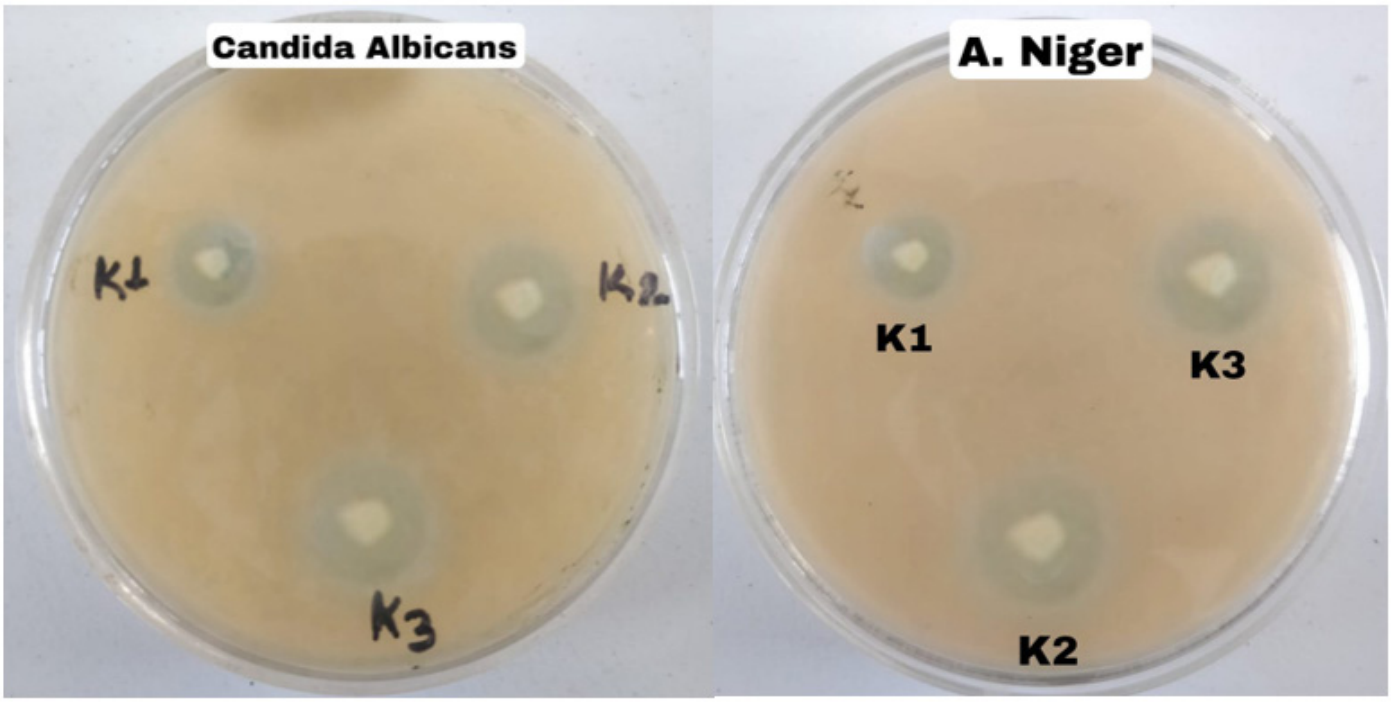


Figure 3: Zone of Inhibition of 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) (*Candida albicans and Aspergillus niger*).

the plates were incubated at 37°C for 48 hr to look for fungus. The MIC was defined as the smallest concentration of the test substance that totally inhibited observable microorganism growth. All tests were carried out in an aseptic environment, and the results were expressed as mean Standard Deviation (SD). One-way Analysis of Variance (ANOVA) was used for statistical analysis, followed by Tukey's multiple comparison tests, with p 0.05 regarded statistically significant.

DISCUSSION

The results showed that most of the synthesized compounds exhibited antifungal activity against *Candida albicans* and *Aspergillus niger* at 25 mg/L,50 mg/L and 100 mg/L. Compound

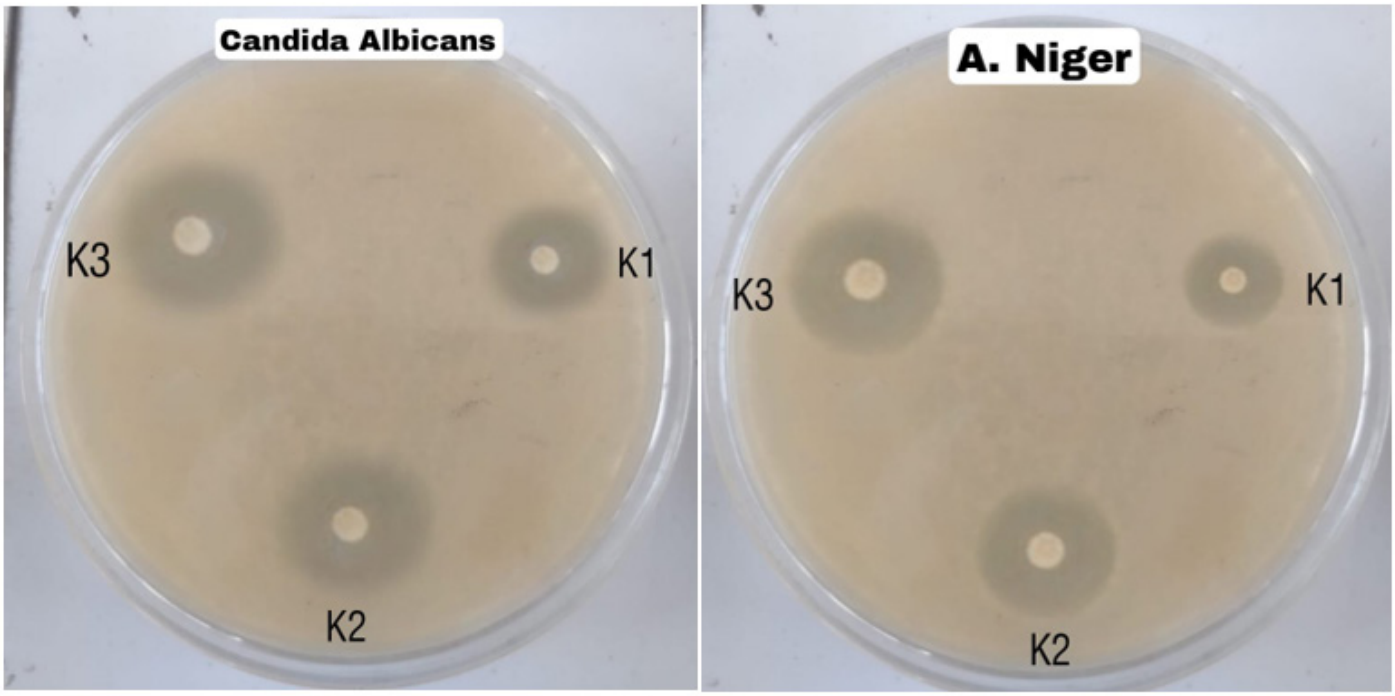


Figure 4: Zone of Inhibition of Griseofulvin (STD)(*Candida albicans and Aspergillus niger*).

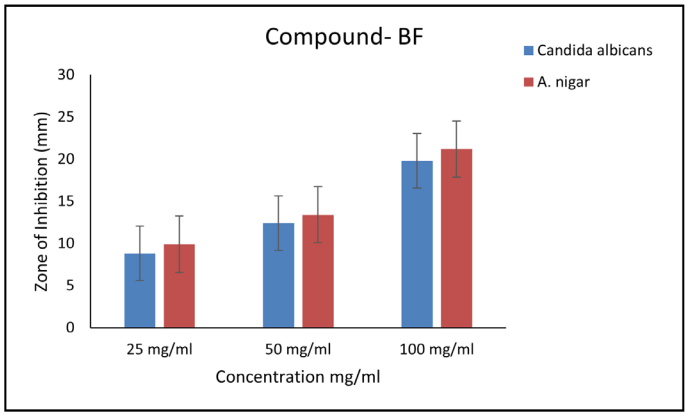


Figure 5: The graphical representation of MIC of sample 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BF) (*Candida albicans* and *Aspergillus niger*).

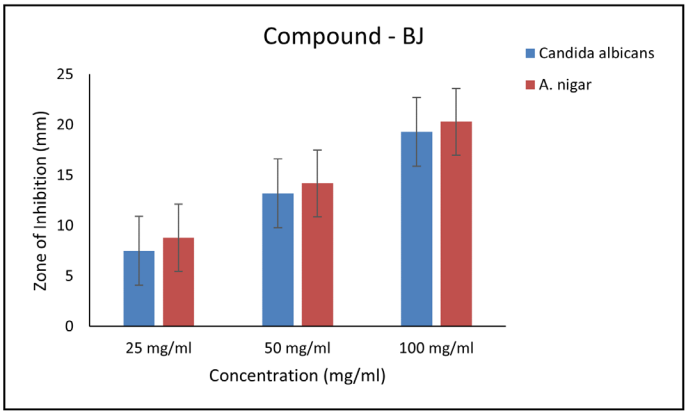


Figure 6: The graphical representation of MIC of sample 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) (*Candida albicans* and *Aspergillus niger*).

BJ, BF displayed higher potent antifungal activity against *Candida albicans* and *Aspergillus niger* with 71% and 65% inhibitory rates, respectively. To the best of our knowledge, this study is the first to report on the anti-fungal activity of 5,5-diphenylimidazolidine-2,4-dione derivatives containing hydantoin moiety. All of the chemicals were synthesised in high yields.[13] The structure of synthesised molecules was confirmed using IR, NMR spectroscopy, and MS. Agar well diffusion method for determining antifungal biological activity. The findings revealed that the majority of the synthesized compounds demonstrated various degrees of inhibition against the *Candida albicans* and *Aspergillus Niger* tested microorganisms.[14] Moreover, the compounds code name like 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ); 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BG); 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BE); 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BK) shown better antifungal activity against *Candida albicans.* 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BF) and 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) shown better antifungal activity against *Aspergillus Niger.* Table 2 shows the preliminary antifungal testing findings of the produced compounds against Griseofulvin. The antifungal activity and Zone of Inhibition of the synthesized drugs were evaluated.[15] A more detailed pharmacological characterization of

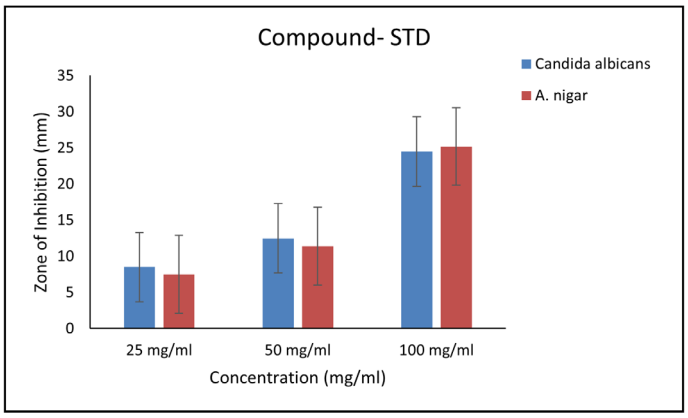


Figure 7: The graphical representation of MIC of sample Griseofulvin (STD) (*Candida albicans and Aspergillus niger*).

compounds, which demonstrated the most beneficial anti-fungal and safety profile, proved its activity in the *in vitro* Anti-microbial Activity by Agar Well Diffusion Method. It should be emphasized that the *in vitro Antimicrobial Activity by Agar Well Diffusion Method* is currently one of the most important screening models for the identification and characterization of novel compounds with a potential efficacy in fungal region. In summary, the obtained *in vivo* data enabled to identify compound code like BJ, BG, BE and BK as a potent and broad-spectrum anti-fungal for future preclinical development (especially through skin administration). It should be emphasized that the key chemical modification performed in the current studies allowed to obtain water-soluble salts which were close analogues of hybrid anti-fungal reported previously. The results of anti-fungal Activity testing of the prepared compounds were shown in Table 2 and Figures 2 to 3. Graphical presentation was shown in Figures 4 to 7. Previously we reported that 5,5-diphenylimidazolidine -2,4-dione derivatives are more potent than Griseofulvin compounds in the point of anti-fungal activity. The results also showed that Aromatic 5,5-diphenylimidazolidine -2,4-dione derivatives are more active than alkyl 5,5-diphenylimidazolidine -2,4-dione derivatives. In the 5,5-diphenylimidazolidine -2,4-dione category 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ); 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BG); 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxo propanoyl]-5,5-diphenylimidazolidine-2,4-dione (BE); 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BK) affected all species of fungi. All tested compounds in this study except 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BF) and 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) showed desirable activity on*Aspergillus niger*. Some of Candida species such as *C. albicans*, which are resistant to fluconazole and itraconazole were affected by the synthesized compounds. In this study compound 1-[3-(oxo (phenyl amino) acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) and 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BG); was affective only on the dermatophytes. Our results showed that anti-fungal activities of the benzimidazole derivatives were optimum with 9 carbon atoms in the alkyl chain and as the number of carbon atoms increases, the antifungal activities will decrease. Compounds 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ); 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BG); 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxo propanoyl]-5,5-diphenylimidazolidine-2,4-dione(BE); 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione(BK) exhibited high inhibitory activity. The results of preliminary SAR study indicated that the fungicidal activity can be decreased by introduction of hydroxyl at the 2-position of the benzene ring, the compound containing a Aromatic NH_2 displayed higher antifungal activity against different fungi than that of benzene,[16] and the substituent of ester and amino group on the phenyl ring can enhance the activities. However, unlike antifungal activity, the free hydroxyl group at the 2-position of the benzene plays an important role in the anti-fungal activity and the compounds containing Carboxylic acid showed much higher activity and the introduction of heterocyclic ring could decrease the anti-fungal activity.[17] Moreover, the methoxy at the 2-position of the benzene ring also improved the antifungal activity. Further studies are currently underway to establish a definite SAR.

CONCLUSION

Griseofulvin, the standard reference compound for this research, it showed the highest activity against all tested micro-organisms at all tested concentrations. At a concentration of 100 mg/mL, Griseofulvin showed moderate to high activity against all 2 micro-organisms with inhibition zones. Various 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]- 5,5-diphenylimidazolidine -2,4-dione (BD) derivatives was synthesized by 1-acetyl-5,5-diphenylimidazolidine -2,4-dione2-hydroxy benzoic acid and 50 mL ethanol into the RBF. The total 11 derivatives of 1-[3-(2-hydroxyphenyl)-3-oxopropanoyl]- 5,5-diphenylimidazolidine -2,4-dione (BD) were synthesized. All of the chemicals were synthesised in high yields. The structure of synthesised molecules was confirmed using IR, NMR spectroscopy, and MS. Agar well diffusion method for determining antifungal biological activity. The compound 1-[3-(oxo (phenyl amino) acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ); 1-[3-(*N*-phenylformamide)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BG); 1-[3-(*N*-Phenyl-2-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BE); 1-[3-(oxo(phenyl-amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BK) shown better antifungal activity against *Candida*

albicans. 1-[3-(*N*-Phenyl-4-nitroaniline)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BF) and 1-[3-(oxo (phenyl amino)acetic acid)-3-oxopropanoyl]-5,5-diphenylimidazolidine-2,4-dione (BJ) shown better anti-fungal activity against *Aspergillus niger* were established to be the most potent compound as compared to standard drugs Griseofulvin.

ACKNOWLEDGEMENT

The authors are thankful to Dr. S.B. Bhawar, Pravara Rural College of Pharmacy, Pravaranagar.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **NMR:** spectroscopy: Nuclear magnetic spectroscopy; **MS:** Mass spectroscopy; **KBr:** Potassium Bromide; **% yield:** Percentage yields; **M.P.:** Melting point; **mg/kg:** Milligram/kilograms; **sec:** Seconds; **δ:** Chemical shift; **Mol.Wt:** Molecular Weight; **g:** Gram.

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Cite this article: Bhor RJ, Shaikh SB, Bapusaheb SR, Bhausaheb SP, Kolhe MH. Synthetic Identification of New Compounds with Anti-fungal Properties of "1-[3-(2-Hydroxyphenyl)-3-Oxopropanoyl]- 5,5-Diphenylimidazolidine -2,4-Dione and its Derivatives". J Young Pharm. 2023;15(4):638-48.