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Synthesis, Characterization and Antimicrobial Activity of Oxadiazine Derivatives

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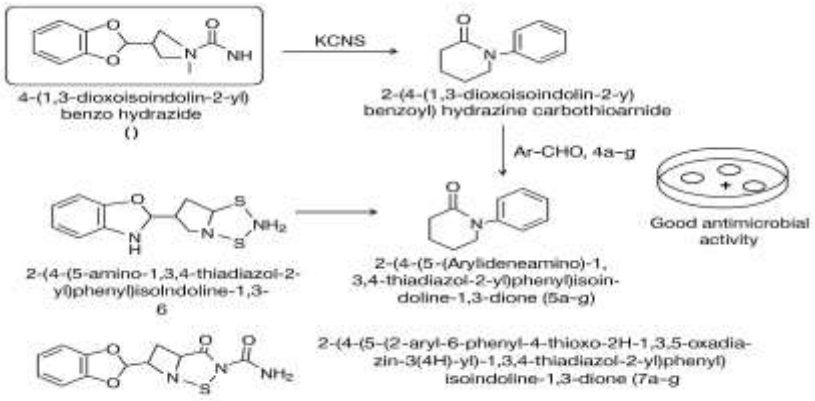
ABSTARCT

4-(1,3-dioxoisoindolin-2-yl)benzo hydrazide **(1)** reacted with KCNS yielded 2-(4-(1,3-dioxoisoindolin-2-yl)benzoyl) hydrazine carbothioamide **(2)**, which on reacted with H_2SO_4 / NH_3 yielded 2-(4-(5-amino-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione(3). The compound **(3)** reacted with various substituted aldehyde**(4a-g)** derivatives yielded 2-(4-(5-(Arylideneamino)-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione **(5a-g)**, they further reacted with benzoyl isothiocyanate **(6)** yielded 2-(4-(5-(2-aryl-6-phenyl-4-thioxo-2H-1,3,5-oxadiazin-3(4H)-yl)-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione **(7a-g)**. All the novel compounds are characterized by elemental analysis a well as spectral study and they confirmed the predicted structure. Antimicrobial Activity of all synthesised compounds are also carried out. All they show moderate to good antimicrobial activity.

GRAPHICAL ABSTRACT



KEYWORDS: Hydrazide, Schiff's base, Oxadiazine, Elemental analysis, Spectral study and Antimicrobial Activity.

INTRODUCTION

The six-member ring system of oxadiazine has one oxygen and two nitrogen atoms; it is a heterocyclic molecule. Oxadiazine demonstrates a wide range of biological and therapeutic actions, including killing bacteria, fungi, HIV, seizures, tumors, inflammation, malaria, tuberculosis, and more [1-10]. They play important role in supramolecular chemistry, medicinal chemistry and as intermediate in organic synthesis

[11-15]. A lot of people are working on synthesizing oxadiazine derivatives right now, and they are showing some really impressive biological and pharmacological activities. Based on the literature review, oxadiazine derivatives are produced by cycloaddition of Schiff's base and benzoyl isothiocyanate [16,17]. looking to the importance of Oxadiazine, authors think to synthesis novel Oxadiazine derivatives, i.e. 2-(4-(5-(2-aryl-6-phenyl-4-thioxo-2H-1,3,5-oxadiazin-3(4H)-yl)-1,3,4-thiadiazol-2-yl) phenyl) isoindoline-1,3-dione **(7a-g)** with good antimicrobial Activity.

EXPERIMENTAL

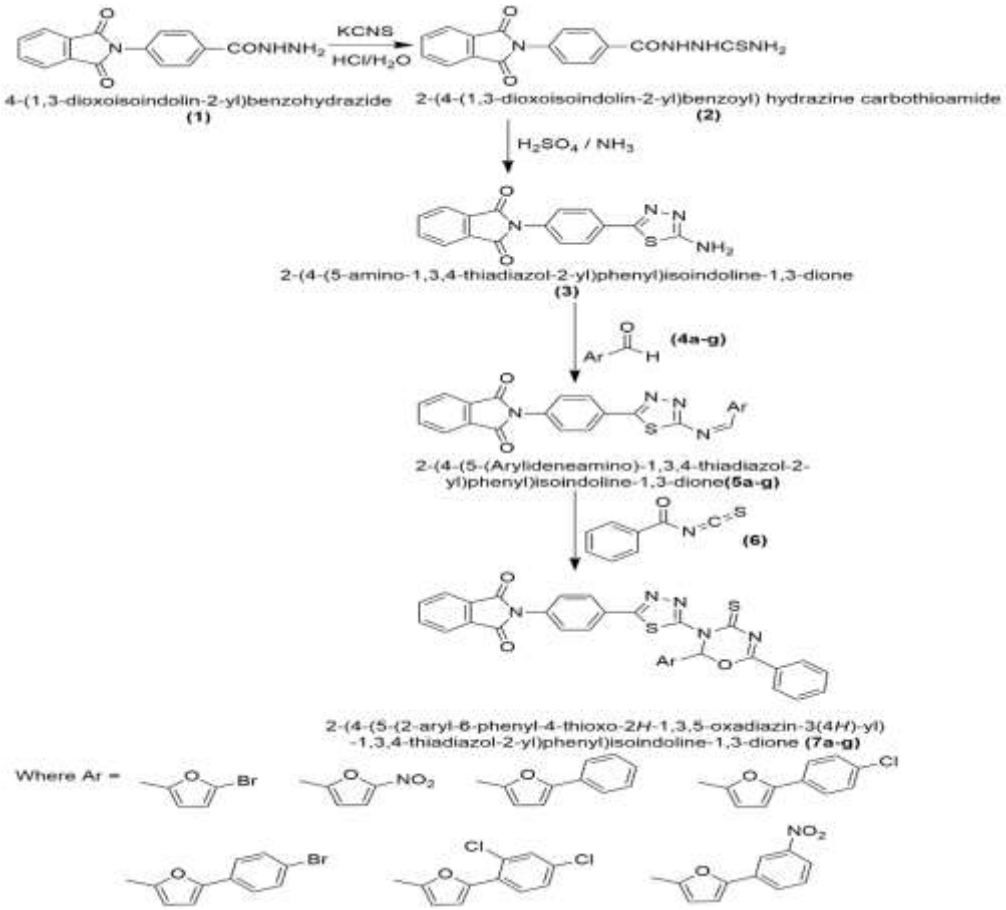
MATERIALS AND METHODS

All the chemicals used were procured as pure grade. 4-(1,3-dioxoisoindolin-2-yl)benzo hydrazide **(1)** was synthesised by reported method [18].

All chemicals' infrared spectra were recorded using a Nicolet 400D spectrometer in KBr pellets. Bruker (400 MHz) spectrometers were used to record proton NMR spectra. There was a solvent utilized, deuterated DMSO. The LC-MSD-Trap-SL_01046 was used to analyze a subset of the samples. Total chromatography (TLC) was used to ensure the chemicals were pure.

Tables 3, 4, and 5 show the results of the antibacterial activity tests conducted on the two series of compounds. The activity was assessed using the agar-cup plate method at a concentration of 50µg/ml. [19-21]. The % age inhibition of growth of bacteria by the compounds is shown in Table-3,4.

Tables 5, 6 reveal the plant pathogens that were chosen for the investigation, and their antifungal activity was assessed in vitro at a concentration of 1000 ppm for both series of compounds [19-21].



Scheme 1 Synthetic route of the preparation of the compound (1), (2), (4a-g), (5a-g), (6) and (7a-g)

Synthesis of 2-(4-(1,3-dioxoisoindolin-2-yl)benzoyl) hydrazine carbothioamide (2)

Add 4-(1,3-dioxoisoindolin-2-yl)benzo hydrazide (1) (0.03mole), 7 mL of concentrated hydrochloric acid, 35 ml of water, and dried potassium thiocyanate (0.04 mole) to a 100 ml round-bottom flask. Set the flask in an oil bath with electromagnetic stirring and heat it to 90–100 degrees Celsius. After one and a half hours, a lot of white solid precipitates. After 3.5 hours of heating and reaction, stop the reaction, cool, filter, wash with water and a diluted sodium bicarbonate solution, and then dry to have a pure product. Yield, m.p. 139-140°C, 67%. A spectral and elemental analysis was used to describe it.

Synthesis of 2-(4-(5-amino-1,3,4-thiadiazol-2-yl) phenyl) isoindoline-1,3-dione (3)

Compound 2-(4-(1,3-dioxoisoindolin-2-yl) benzoyl) hydrazine carbothioamide (2) (0.01 mole) and con. H_2SO_4 (0.01 mole) were equimolarly mixed in 15 ml of methanol and left at room temperature overnight. For almost 7.5 hours, it was refluxed in a steam bath. The reaction mixture was cooled, neutralized with concentrated liquid ammonia, and then filtered. After the solvent was eliminated using vacuum, the resulting solid was dried and purified over a silica gel column using an eluent consisting of a chloroform: methanol (5:5 v/v) mixture. Compound 3 was obtained by recrystallizing the product from ethanol after the eluate was concentrated [22]. 71% yield, m.p. 168–169°C. Spectral and elemental analysis were used to describe it.

Synthesis of 2-(4-(5-(Arylideneamino)-1,3,4-thiadiazol-2-yl) phenyl) isoindoline-1,3-dione (5a-g)

For almost two and a half hours, an equimolar mixture of compound 3 (0.01 mole) and several substituted aldehydes (4a-g) (0.01 mole) in 50 milliliters of methanol with four to five drops of glacial acetic acid was refluxed on a water bath. The resulting solid was purified over a silica gel column using a chloroform: methanol (6:4 v/v) mixture as an eluent after the solvent was distilled out at a lower pressure. Crystals of the chemical (4a) were obtained by recrystallizing the product with ethanol after the eluate had been concentrated [22]. Using compound 3 and several substituted aldehydes (4b-g), another molecule (5b-g) was produced in a similar way. The elemental analysis, yield, and m. p. are shown in Table 1.

Table-1 Analysis of compounds (5a-g)

Comp. No.	Molecular Formula	M.P.* ˚C	Elemental Analysis				
			C%	H%	N%	S%	X%
			Calcd. (Found)	Calcd. (Found)	Calcd. (Found)	Calcd. (Found)	Calcd. (Found)
5a	$C_{21}H_{11}N_4O_3SBr$ (477)	176-177	52.62 52.6	2.31 2.3	11.69 11.6	6.69 6.6	16.67 16.6
5b	$C_{21}H_{11}N_5O_5S$ (445)	192-193	56.63 56.6	2.49 2.4	15.72 15.7	7.20 7.1	-
5c	$C_{27}H_{16}N_4O_3S$ (476)	185-186	68.06 68.0	3.38 3.3	11.76 11.7	6.73 6.7	-
5d	$C_{27}H_{15}N_4O_3SCl$ (510.5)	168-169	63.47 63.4	2.96 2.9	10.97 10.9	6.28 6.8	6.94 6.9
5e	$C_{27}H_{15}N_4O_3SBr$ (554)	179-180	58.39 58.3	2.72 2.7	10.09 10.0	5.77 5.7	14.39 14.3
5f	$C_{27}H_{14}N_4O_3SCl_2$	186-187	59.46	2.59	10.27	5.88	13.00

	(544)		59.4	2.5	10.2	5.88	12.9
5g	$C_{27}H_{15}N_5O_5S$ (521)	198-199	62.18 62.1	2.90 2.8	13.43 13.4	6.15 6.1	-

* Uncorrected. LC-MS data for 5e:557.3

Synthesis of 2-(4-(5-(2-aryl -6-phenyl -4-thioxo -2H-1,3,5-oxadiazin-3(4H)-yl)-1,3,4-thiadiazol -2-yl) phenyl)isoindoline-1,3-dione (7a-g)

A combination of 2-(4-(5-(Arylideneamino)-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione (5a-g) (0.01 mol), benzoyl isothiocyanate (6) (0.01 mol), and triethylamine (2-3 drops) in 1,4-dioxane (25 mL) was subjected to reflux for 2.5 hours. The precipitate obtained from dilution with 30 mL of water was filtered, dried, and recrystallized from xylene to yield crystals of the product (7a-g) [23]. The other compounds (7b-g) were produced similarly utilizing compound (6) and different derivatives (5b-g). The yield, melting point, and elemental analysis are presented in Table 2.

Table 2. Analysis of compounds (7a-g)

Comp. No.	Molecular Formula	M.P.* ˚C	Elemental Analysis				
			C%	H%	N%	S%	X%
			Calcd. (Found)	Calcd. (Found)	Calcd. (Found)	Calcd. (Found)	Calcd. (Found)
7a	$C_{29}H_{16}N_5O_4S_2Br$ (642)	189-190	54.21 54.2	2.51 2.5	10.90 10.8	9.98 9.9	12.44 12.4
7b	$C_{29}H_{16}N_6O_6S_2$ (608)	205-206	57.23 57.2	2.65 2.6	13.81 13.8	10.54 10.5	-
7c	$C_{35}H_{21}N_5O_4S_2$ (639)	213-214	65.71 65.7	3.31 3.3	10.95 10.9	10.02 10.0	-
7d	$C_{35}H_{20}N_5O_4S_2Cl$ (673.5)	207-208	62.36 62.3	2.99 2.9	10.39 10.3	9.51 9.5	5.26 5.2
7e	$C_{35}H_{20}N_5O_4S_2Br$ (717)	203-204	58.50 58.4	2.81 2.8	9.75 9.7	8.92 8.9	11.12 11.1
7f	$C_{35}H_{19}N_5O_4S_2Cl_2$ (707)	210-211	59.33 59.3	2.70 2.6	9.88 9.8	9.05 9.0	10.01 10.0
7g	$C_{35}H_{20}N_6O_6S_2$ (684)	218-219	61.40 61.3	2.94 2.9	12.27 12.2	9.37 9.3	-

* Uncorrected. LC-MS data for 7e:717.5

RESULTS AND DISCUSSION

The 2-(4-(1,3-dioxoisoindolin-2-yl)benzoyl) hydrazine carbothioamide **(2)** was synthesised from 4-(1,3-dioxoisoindolin-2-yl)benzo hydrazide **(1)** on reaction with potassium thiocyanate. The elemental characterization of compound **(2)** shows mol. formula: $C_{16}H_{12}N_4O_3S$ (340 gm/mole) : C, 56.46, H, 3.55, N, 16.46,S,9.42%, found : C, 56.4, H, 3.5, N, 16.4,S,9.4%; IR(υ,cm^{-1}): 3410, 3268 (-NH_2), 3356 (-NH), 3080-3000 (C–H aromatic st.), 1782-1648 (C=O), 1428 (N-C=O), 1160 (-C=S), 1120(C-N);1HNMR DMSO–d_6, (δ,ppm): 8.63 (s, 2H, -NH_2), 7.94-8.00 (s, 2H, -NH), 7.92-7.40 (m, 8H, Ar-H).

The 2-(4-(5-amino-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione **(3)** was synthesised from 2-(4-(1,3-dioxoisoindolin-2-yl)benzoyl) hydrazine carbothioamide **(2)** on reaction with con. H_2SO_4 followed by liq. Ammonia. The elemental characterization of compound **(3)** shows mol. formula: $C_{16}H_{10}N_4O_2S$(322gm/mole) : C, 59.62, H, 3.13, N, 17.38,S, 9.95 %, found : C, 59.6, H, 3.1, N, 17.3,S,9.9; IR(υ,cm^{-1}): 3286, 3196(-NH_2), 3070-2999(C–H aromatic st.) 1627 (-N=C-S),1601(C-N), 1203 (C-S), 1782-1648 (C=O), 1428 (N-C=O), 1120(C-N); 1HNMR DMSO–d_6, (δ,ppm): 4.25 (s, 2H, -NH_2) and 8.06-7.35 (m, 8H, Ar-H).

The compound **(3)** react with various substituted aldehydes **(4a-g)** yielded 2-(4-(5-(Arylideneamino)-1,3,4-thiadiazol-2-yl)phenyl)isoindoline-1,3-dione **(5a-g)**. Their yield,m.p. and elemental analysis shown in Table-1.Its IR (υ,cm^{-1}): 3080-3000 (C–H aromatic st.) 1625 (-N=C-S),1600(C-N), 1200 (C-S), 1783-1646 (C=O), 1428 (N-C=O), 1120(C-N), 1548 (-N=CH),1280(C-O),685(C-Br),1543,1368 (NO_2), 1082(C-Cl); 1HNMR DMSO–d_6, (δ,ppm): 8.05-7.38 (m, 8H, Ar-H), 8.92 (s, 1H, N=CH-Ar); (a)7.10-6.85(m,2H,furan-H), (b) 7.65-7.02(m,2H,furan-H), (c) 7.10-6.56(m,2H, furan -H), 8.12 -7.45(m,5H,Ar-H), (d) 7.10-6.56(m,2H, furan -H),7.80-7.58(m,4H,Ar-H), (e) 7.12-6.58 (m, 2H, furan -H),7.70-7.63(m,4H,Ar-H), (f) 7.10-6.58(m,2H, furan -H),7.78-7.45 (m,3H,Ar-H), (g) 7.10-6.58(m,2H, furan -H),8.75-7.78(m,4H,Ar-H).

The compound **(5a-g)** further react with benzoyl isothiocyanate **(6)** yields 2-(4-(5-(2-aryl -6-phenyl-4-thioxo -2H-1,3,5-oxadiazin-3(4H)-yl)-1,3,4-thiadiazol -2-yl) phenyl)isoindoline-1,3-dione **(7a-g)**. Their yield and elemental analysis shown in Table-1.The IR (υ,cm^{-1}): 3085-3005 (C–H aromatic st.) 1628-1620 (-N=C-S),1605(C-N), 1210 (C-S), 1783-1646 (C=O), 1428 (N-C=O),1120(C-N), 1280(C-O), 1160 (-C=S), 685(C-Br),1543,1368 (NO_2), 1082(C-Cl); 1HNMR DMSO–d_6, (δ,ppm): 8.05-7.38 (m, 13H, Ar-H), 5.92 (s, 1H, Oxadiazine-H); (a)6.50-6.45(m,2H,furan-H), (b) 7.60-7.02(m,2H,furan-H), (c) 7.08-6.55(m,2H, furan -H), 8.10 -7.42(m,5H,Ar-H), (d) 7.10-6.52(m,2H, furan -H),7.78-7.55(m,4H,Ar-H), (e) 7.10-6.56 (m, 2H, furan -H),7.68-7.60(m,4H,Ar-H), (f) 7.10-6.55(m,2H, furan -H),7.76-7.40 (m,3H,Ar-H), (g) 7.10-6.55(m,2H, furan -H),8.72-7.75(m,4H,Ar-H),

Table 3. Antibacterial Activity of Compounds (5a-g)

Comp. No.	***Zone of Inhibition(mm)***			
	Gram +ve		***Gram -ve***	
	Bacillus Subtilis	***Staphylococcus aureus***	***Klebsiella promioe***	***Escherichia coli***
5a	07	06	06	07
5b	08	07	06	08
5c	10	08	08	09
5d	12	11	11	12
5e	11	10	10	11
5f	17	16	15	16
5g	15	13	13	14
Tetracycline	20	19	18	22

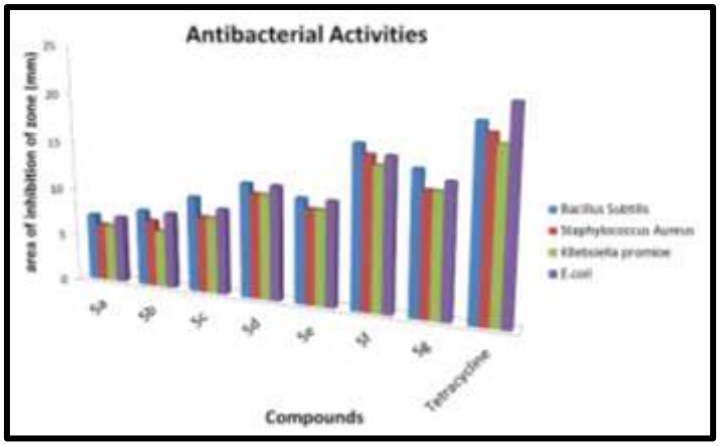


Figure 1 Antibacterial Activities of Synthesis compound (5a-5g)

Table 4. Antibacterial Activity of Compounds (7a-g)

Comp. No.	*Zone of Inhibition(mm)*			
	Gram +ve		*Gram -ve*	
	Bacillus Subtilis	*Staphylococcus aureus*	*Klebsiella promioe*	*Escherichia coli*
7a	09	07	08	09
7b	10	09	09	09
7c	12	10	10	10
7d	13	12	12	13
7e	12	11	10	12
7f	18	17	16	17
7g	16	15	14	15
Tetracycline	20	19	18	22

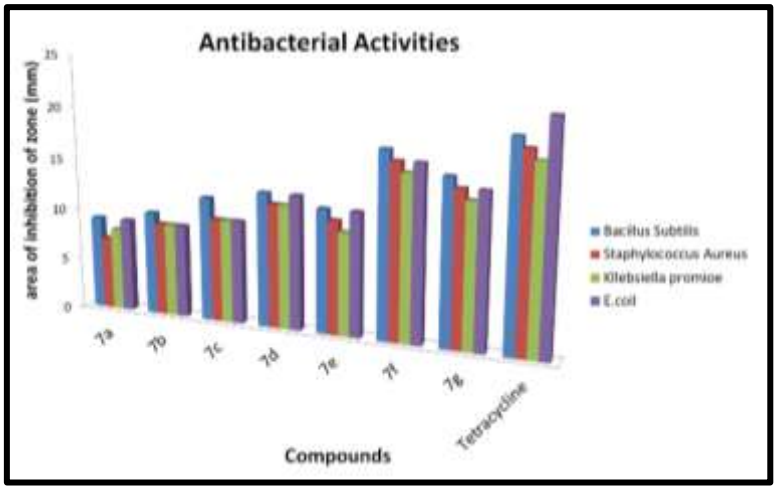


Figure 2 Antibacterial Activies of synthesis compound (7a-7g)

All elemental and spectral characteristics indicate that the data align with the anticipated structure depicted in Scheme-1. The LC-MS analysis of chosen chemicals reveals a peak corresponding to the M+ ion, consistent with their molecular weight. All these data validate the structures (5a-g) and (7a-g).

Table 5. Antifungal Activity of Compounds (5a-g)

Comp. No.	*Zone of Inhibition at 1000 ppm (%)*			
	Botryodiplodia theobromae	*Nigrospora Spp.*	*Penicillium Expansum*	*Rhizopus Nigricans*
5a	62	61	60	53
5b	65	66	63	54
5c	66	65	64	56
5d	69	70	67	60
5e	68	67	65	58
5f	75	73	71	66
5g	72	71	69	63

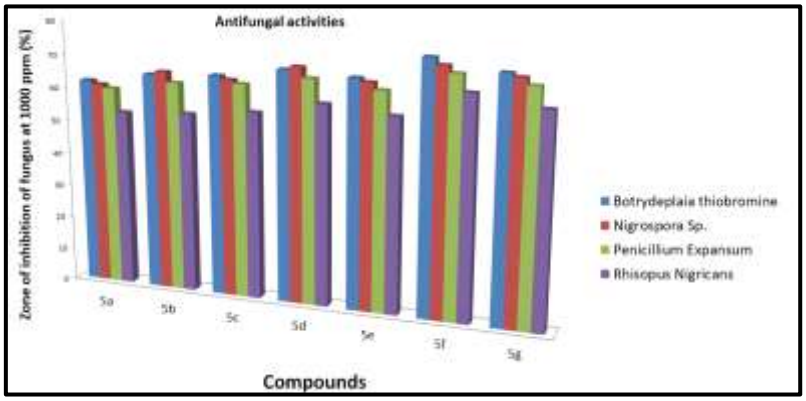


Figure 3 Antifungal Activity of Synthesis Compound (5a-5g)

Table 6. Antifungal Activity of Compounds (7a-g)

Comp. No.	*Zone of Inhibition at 1000 ppm (%)*			
	Botryodiplodia theobromae	*Nigrosspora Spp.*	*Penicillium Expansum*	*Rhizopus Nigricans*
7a	65	62	62	54
7b	68	68	64	55
7c	69	69	65	58
7d	73	72	69	62
7e	71	72	68	62
7f	79	76	73	70
7g	73	74	70	65

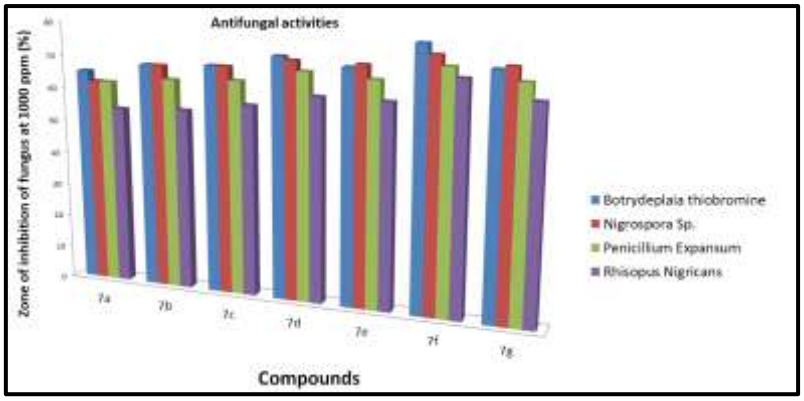


Figure 4 Antifungal Activity of Synthesis compound (7a-7g)

The analysis of antibacterial and antifungal activity data indicates that all compounds exhibit toxicity towards microbes, with compounds 7f, 7g, and 5f demonstrating heightened efficacy against both gram-positive and gram-negative bacteria, as well as fungi.

CONCLUSION

A series of novel isoindoline–thiadiazole derivatives were successfully synthesized through a stepwise approach starting from 4-(1,3-dioxoisoindolin-2-yl)benzo hydrazide. The synthesized compounds (2–7a-g) were characterized by elemental analysis, IR, ^{1}H NMR, and LC-MS, all of which confirmed the proposed structures. The spectral data corresponded well with the anticipated functional groups and molecular formulas, validating the synthetic pathway. These findings establish a reliable route for the development of thiadiazole-based heterocycles, which may serve as promising scaffolds for further biological evaluation.

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