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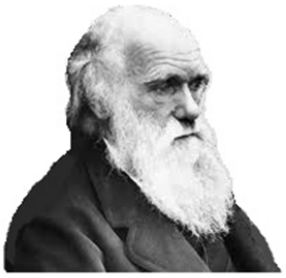

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Synthesis, spectral studies and antimicrobial activity of newly chalcones and isoxazoles in diphenyl amine derivatives

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ABSTRACT

A new series of compounds, namely 2-{[4'-(3"-aryl)-2"-propene-1"-one-phenyl]amino}benzoic acids (3a-3j) and 2-{[4'-(5"-aryl)isoxazol-3"-yl]phenyl amino} benzoic acids (4a-4j), were synthesized. The structures of these compounds were confirmed by ^{1}H NMR, IR, Mass spectral analysis. The compounds (3a-3j) and (4a-4j) have been evaluated their antimicrobial activity.

***Keywords*:** Chalcones, isoxazoles, Anti-bacterial activity, Anti-fungal activity, Heterocyclic compounds

1. INTRODUCTION

Chalcone are a type of organic compounds that belongs to the flavonoid family [1]. They consist of two aromatic rings connected by a three-carbon α,β-unsaturated carbonyl system [2]. Chalcones have industrial importance and are also used in the medicinal field. Chalcones are use to synthesized Pyrazoline, Pyrimidine, Pyrazole, and Thiosemicarbazide derivatives because of highly reactive unsaturated pi-bond [3]. Existing data shows that nitrogen-containing heterocyclic compounds such as isoxazole have medicinal importance because of their biological activities [4]. Isoxazole are another class of important heterocyclic compounds containing a five-membered ring with one nitrogen atoms, one oxygen atom and three carbon

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atoms [5-6]. The combination of chalcone and isoxazole moieties allows medicinal chemists to create hybrid molecules with a broad range of biological activities [7-8].

Chalcones and isoxazoles have been found to exhibit various biological activity, such as Antibacterial [9-10], Antifungal [11-12], Antitumor [13], Anti-inflammatory [14], Anthelmintic [15], Anticancer [16], Anti-HIV [17], Antihypertensive [18], Antihistaminic [19], Anti-oxidan t [20], Anti-convulsant [21] and Analgesic [22] activities.

Our research work focuses on synthesized heterocyclic compounds derived from chalcones, which exhibit many biological activities and find various applications in industries. In our study, we have dedicated our efforts to synthesized, of isoxazole derivatives (4a-4j) derived from chalcones (3a-3j). Subsequently, we evaluated the antimicrobial properties of these newly synthesized chalcone and isoxazole derivatives and compared their activity with known standard drugs using the cup-plate method [23-26].

The cup-plate method is commonly used to assess the antimicrobial activity of synthesized compounds. This method depends on the diffusion of an antibiotic from a vertical cavity, through the agar layer containing microorganisms in a petri plate. In this method, prepare an agar plate, and a swap of pure bacterial culture is evenly spread on the agar plate. Then, the synthesized compounds are added to the agar plate containing microorganisms, now kept this petri plate for incubation for 24 to 30 hours to allow them to diffuse and come into contact with the microorganisms. After an incubation period, a clear area (Zone of inhibition) around the tested sample is observed and measured. This measured zone indicates the antimicrobial activity of the compounds. Zone of inhibition measured in mm.

2. MATERIALS AND METHODS

The synthesis process involved the use of analytical grade (AR) chemicals sourced from SRL and Phenar companies, which were employed without any additional purification. All reactions took place under specified conditions. The purity of the synthesized compounds was assessed using TLC with silica gel G (Merk) and the solvent system ethyl acetate: hexane (2:3). The TLC plates were visualized under UV at 260 nm. Characterization of the compounds was performed through spectral analysis, including MS (Mass Spectrometry), IR, and ^{1}H-NMR. Mass spectra were recorded using a water Mass spectrometer. Infrared spectroscopy was conducted using KBr on a Shimadzu IR Affinity FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO solvent with TMS as an internal standard. Melting points of the synthesized compounds were determined in open glass capillary tubes and are uncorrected. The antimicrobial activities, of the synthesized compounds (3a-3j), (4a-4j) have been taken by the Cup plate method, with known standard drugs utilized for comparison.

General preparation: 2-{4'-[3''-(3''',4'''-dimethoxyphenyl)-2''-propene-1''-one-phenyl] amino}benzoic acid. (3a)

To a solution containing 2-[(4'-acetylphenyl)amino]benzoic acid (0.01 m) in methanol, an appropriate 3,4-dimethoxy benzaldehyde (0.01 m) was added. A catalytic amount of 40% NaOH solution was then introduced, and the resulting reaction mass was stirred at RT for 24 hrs. The reaction is monitored by (TLC). After completion of the reaction, the reaction mass was poured ice and add con. HCl and filtered, dry it. The obtained product was crystalized in

methanol, resulting to the formation of the desired compounds. Yield: 93%; M.P.: 177 °C, ^{1}H NMR (400 MHz, DMSO), δ 9.84 (s, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 7.97 (d, 1H), 7.92 – 7.81 (m, *J* = 30.9 Hz, 1H), 7.81 – 7.66 (m, 1H), 7.65 – 7.54 (m, 1H), 7.55 – 7.46 (m, *J* = 11.6, 9.9 Hz, 1H), 7.43 – 7.33 (m, 1H), 7.31 – 7.23 (m, *J* = 13.3, 6.0 Hz, 1H), 7.23 – 7.09 (m, *J* = 16.7, 8.4 Hz, 1H), 7.08 – 6.90 (m, 1H), 6.82 (dd, *J* = 13.1, 5.8 Hz, 1H), 6.62 (dd, *J* = 8.7 Hz, 1H), 6.13 (d, 1H), 3.85 (s, 3H), 3.82 (s, 3H). IR (cm^{-1}): 2927 (C-H Str. Aromatic), 2927 (C-H Str. Alkane), 1452-1334 (C-H def. Alkane), 1579 (C=C Str. Aromatic), 1292 (C-H Def. Aromatic), 1633 (C=O Str.), 1633 (CH=CH Str. Vinyl), 1539 (C-O-C Str.), 1633 (N-H Str.), 1259 (O-H). MS: at M/Z = 403, Anal. Calc. for $C_{24}H_{21}NO_5$; C: 71.45%, H: 5.25%, N: 3.47%, O: 19.83% Found C: 71.16%, H: 5.00%, O: 19.68%, N: 3.36%.

Similarly other (3a-3j) compounds have been synthesized.

Reaction scheme 1:

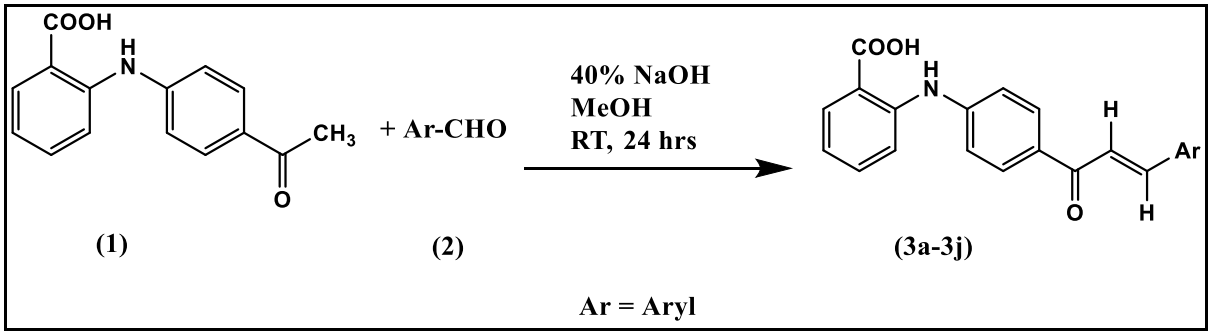

Table 1. Physical and analytical data of 2-{[4'-(3"-aryl)-2"-propene-1"-one-phenyl]amino} benzoic acids. (3a-3j)

Sr. no	Ar-	M.F	M.W	M.P	%Yield	% of nitrogen	
						Calc.	Found
3a	3,4-$(OCH_3)_2$-C_6H_3-	$C_{24}H_{21}NO_5$	403	177	93	3.47	3.36
3b	4-OH,3-OCH_3-C_6H_3-	$C_{23}H_{19}NO_5$	389	180	89	3.60	3.57
3c	C_6H_5-	$C_{22}H_{17}NO_3$	343	140	78	4.08	4.00
3d	4-OCH_3-C_6H_4-	$C_{23}H_{19}NO_4$	373	160	86	3.75	3.65
3e	4-OH-C_6H_4-	$C_{22}H_{17}NO_4$	359	187	75	3.90	3.88
3f	2-NO_2-C_6H_4-	$C_{22}H_{16}N_2O_5$	388	170	70	7.21	7.10
3g	3-NO_2-C_6H_4-	$C_{22}H_{16}N_2O_5$	388	175	79	7.21	7.13
3h	4-Cl-C_6H_4-	$C_{22}H_{16}ClNO_3$	377	150	84	3.71	3.66

3i	4-Br-C_6H_4-	$C_{22}H_{16}BrNO_3$	421	190	83	3.32	3.21
3j	3,4,5-$(OCH_3)_3$-C_6H_2-	$C_{25}H_{23}NO_6$	433	195	73	3.23	3.20

General preparation: 2-{4'-[5''-(3''',4'''-dimethoxyphenyl)isoxazol-3''-yl]phenyl amino} benzoic acid. (4a)

To a solution of 2-{4'-[3"-(3"',4"'-dimethoxyphenyl)-2"-propene-1"-one-phenyl]amino} benzoic acid (0.01 m) and hydroxyl amine hydrochloride (0.02 m) in methanol add small amount of alcoholic NaOH and reflux it for 8hrs at 80 °C. The reaction is monitored by (TLC). After the reaction completion, the reaction mass was poured into maltreated ice and filtered, dry it. The obtained product was crystalized in methanol, resulting to the formation of the desired compounds. Yield: 86%; M.P.: 170 °C, ^{1}H NMR (400 MHz, DMSO), δ 13.16 (s, 1H), 9.74 (s, 1H), 7.97 – 7.90 (m, 1H), 7.68 – 7.61 (m, *J* = 8.6 Hz, 1H), 7.51 – 7.44 (m, *J* = 12.7, 8.6 Hz, 2H), 7.43 – 7.39 (m, 1H), 7.36 – 7.32 (m, 1H), 7.32 – 7.26 (m, *J* = 16.5 Hz, 1H), 7.26 (dd, *J* = 8.7 Hz, 1H), 7.12 (dd, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 6.84 (d, 1H), 3.81 (s, 3H), 3.77 (s, 3H). IR (cm^{-1}), 3387 (C-H Str. Aromatic), 2945 (C-H Str. Alkane), 1462-1311 (C-H def. Alkane), 1516 (C=C Str. Aromatic), 1294 (C-H Def. Aromatic), 1649 (C=O Str.), 1423 (CH=CH Str. Vinyl), 1244 (C-O-C Str.), 974 (C-F Str.), 1006 (C-N Str.), 1587 (C=N Str.), 3504 (N-H Str.). MS: at M/Z = 416. Anal. Calc. for $C_{24}H_{20}N_2O_5$; C: 69.22%, H: 4.84%, N: 6.73%, O: 19.21%. Found C: 68.81%, H: 4.00%, N: 6.66%, O: 19.00%.

Similarly other (4a-4j) compounds have been synthesized.

Reaction scheme 2:

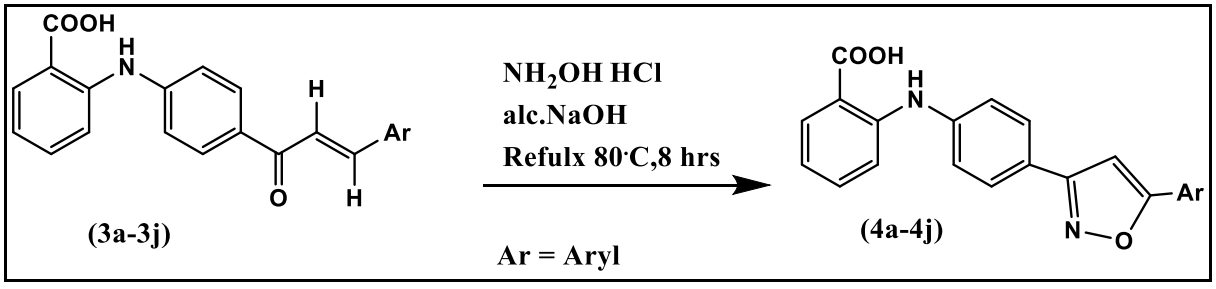

Table 2. Physical and analytical data of 2-{[4'-(5"-aryl)isoxazol-3"-yl]phenyl amino} benzoic acids. (4a-4j)

Sr. no	Ar-	M.F	M.W	M.P	%Yield	% of Nitrogen	
						Calc.	Found.
4a	3,4-$(OCH_3)_2$-C_6H_3-	$C_{24}H_{20}N_2O_5$	416	170	86	6.73	6.66
4b	4-OH,3-OCH_3-C_6H_3-	$C_{23}H_{18}N_2O_5$	402	185	74	6.93	6.90

4c	C_6H_5-	$C_{22}H_{16}N_2O_3$	356	150	88	7.82	7.80
4d	4-OCH_3-C_6H_4-	$C_{23}H_{18}N_2O_4$	386	167	90	7.21	7.20
4e	4-OH-C_6H_4-	$C_{22}H_{16}N_2O_4$	372	195	87	7.48	7.45
4f	2-NO_2-C_6H_4-	$C_{22}H_{15}N_3O_5$	401	177	78	10.42	10.40
4g	3-NO_2-C_6H_4-	$C_{22}H_{15}N_3O_5$	401	160	73	10.42	10.42
4h	4-Cl-C_6H_4-	$C_{22}H_{15}ClN_2O_3$	390	155	82	7.13	7.11
4i	4-Br-C_6H_4-	$C_{22}H_{15}BrN_2O_3$	434	180	70	6.41	6.38
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	$C_{25}H_{22}N_2O_6$	446	190	64	6.25	6.22

3. RESULT AND DISCUSSION

3. 1. Antimicrobial activity

The antibacterial and antifungal activity is done by the cup plate method through zone of inhibition in mm. The concentration of the sample & standard drug is 50 µg/ml using DMSO as solvent. The anti-bacterial activity was taken by Gram-positive bacteria *Bascillis subtilis, Staphylococcus aureus* and Gram-negative bacteria *proteus vulgaris, Escherichia coli*. The anti-fungal activity was taken by *Aspergillus niger* fungus. The antimicrobial activity compared with known standard drugs Streptomycin, Ampicillin, Tetracyclin and Nystatin. The Zone of inhibition was measured in mm. Were Zone of inhibition of compounds (3a-3j) & (4a-4j) are shown in the Table No. 3 and 4. Comparable antimicrobial activity represent in Table No. 5.

Table 3. Antimicrobial activity data of 2-{[4'-(3"-aryl)-2"-propene-1"-one-phenyl]amino} benzoic acids. (3a-3j)

Sr. No.	**Ar-**	**Antibacterial activity, zone of inhibition in m.m.**				**Antifungal activity, Zone of inhibition m.m.**
		Gram-positive bacteria		**Gram-negative bacteria**		
		B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*A. niger*
3a	3,4-$(OCH_3)_2$-C_6H_3-	22	18	18	12	6
3b	4-OH,3-OCH_3-C_6H_3-	21	20	19	11	6
3c	C_6H_5-	22	18	16	12	6

3d	4-OCH_3-C_6H_4-	24	20	18	10	8
3e	4-OH-C_6H_4-	18	20	15	12	13
3f	2-NO_2-C_6H_4-	20	21	17	13	10
3g	3-NO_2-C_6H_4-	20	23	13	14	11
3h	4-Cl-C_6H_4-	21	23	14	13	9
3i	4-Br-C_6H_4-	18	23	8	11	9
3j	3,4,5-$(OCH_3)_3$-C_6H_2-	20	22	16	15	8

Table 4. Antimicrobial activity data of 2-{[4'-(5"-aryl)isoxazol-3"-yl]phenyl amino} benzoic acids. (4a-4j)

Sr. No.	**Ar-**	**Antibacterial activity, zone of inhibition in m.m.**				**Antifungal activity, Zone of inhibition m.m.**
		Gram-positive bacteria		**Gram-negative bacteria**		
		B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*A. niger*
4a	3,4-$(OCH_3)_2$-C_6H_3-	19	20	11	14	12
4b	4-OH,3-OCH_3-C_6H_3-	22	20	15	13	15
4c	C_6H_5-	18	23	15	12	9
4d	4-OCH_3-C_6H_4-	16	21	14	10	11
4e	4-OH-C_6H_4-	14	22	14	15	14
4f	2-NO_2-C_6H_4-	12	20	13	14	15
4g	3-NO_2-C_6H_4-	24	22	18	13	8
4h	4-Cl-C_6H_4-	23	22	18	12	7
4i	4-Br-C_6H_4-	23	21	17	13	9
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	22	20	19	12	7

Table 5. Compounds (3a-3j), (4a-4j) showing antibacterial & antifungal activity compared with known standard drugs.

Compound No.	Antibacterial activity, zone of inhibition in mm.				Antifungal activity, zone of inhibition in mm.
	Gram-positive bacteria		Gram-negative bacteria		
	B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*A. niger*
(3a-3j)	3a,3b,3c,3d,3h	3g,3h,3i,3j	3b	3f,3g,3h,3j	-
(4a-4j)	4a,4g,4h,4i,4j	4c,4d,4e,4g.4h,4i	4e,4g,4h,4j	4a,4e,4f	4a,4e
Activity of known standard drugs:					
Drugs	*B. subtilis*	*S. aureus*	*E. coli*	*P. vulgaris*	*A. niger*
Streptomycin	26	27	28	20	0
Ampicillin	25	26	26	19	0
Tetracycline	25	26	27	19	0
Nystatin	0	-	-	-	22

4. CONCLUSION

We have synthesized chalcones (3a-3j) 2-{[4'-(3"-aryl)-2"-propene-1"-one-phenyl] amino}benzoic acids. The isoxazole derivatives (4a-4j) 2-{[4'-(5"-aryl)isoxazol-3"-yl]phenyl amino} benzoic acids**.** The structure of compounds confirmed by ^{1}H-NMR , IR, Mass spectra. the synthesized compounds were subjected to antibacterial & antifungal activities. Compounds **3a, 3b, 3c, 3d, 3g, 3h, 3i, 3j** and **4a, 4c, 4d, 4e, 4g, 4h, 4i, 4j** exhibited significant antibacterial activity against Gram-positive bacteria as compared to known standard drugs and Compounds **3b, 3 f, 3g, 3h, 3i** and **4a, 4c, 4d, 4e, 4f, 4g, 4h, 4j** give remarkable activity against Gram-negative bacteria as compared to known standard drugs and Compounds **4a, 4e** displayed good antifungal activity as compared to known standard drugs with the same concentration 50 µg/ml.

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