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DESIGN, CONVENTIONAL AND MICROWAVE ASSISTED SYNTHESIS OF MOLECULAR HYBRIDS OF THIAZOL-2-AMINE CONTAINING FLAVONE / CHALCONE MOIETIES POSSESSING ANTIMICROBIAL PROPERTIES

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ABSTRACT
Some thiazol-2-amino-chalcone hybrids and thiazol-2-amino-flavones hybrids have been designed, synthesized and characterized the structure by FT-IR, H^1-NMR and Mass spectra. Their *invitro* antimicrobial effect was evaluated, revealing that chalcone hybrids with thiazol-2-amine (**10a-d**) have the potential antibacterial activities against all the screened four Gram Positive and Gram-negative bacteria as well as four fungal strains. Compound (**10a**) demonstrate Minimum Inhibitory Concentration (MIC) activities against *E.Coli*, *K. Pneumoniae*, *S. albus* and *B. subtilis* at 6.25 µg/mL. Compounds (**10c & d**) exhibit significant MIC activities against *S. albus*, *B. subtilis* and *A. fumigates* at 6.25 µg/mL. Among the synthesized compounds, chalcone bearing 2-amino-thiazole derivatives (**10a-d**) showed better antimicrobial activity (>50%) compare with flavone bearing 2-amino-thiazole derivatives (**8a-d**). Compound **8a**, displayed equipotent antifungal activity as that of standard Clotrimazole and MIC at 12.5 µg/mL against C. *albicans*.

KEYWORDS: Flavones, Chalcone, 2-Amino Thiazoles and Antimicrobial

1. INTRODUCTION
In the past 60 years, antibiotics have been critical in the fight against infectious disease caused by bacteria and other microbes. The rapid development of bacterial strains resistant to antibacterial agents poses a significant threat to global health.[1] Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture. Despite the many antibiotics and chemotherapeutics available, the emergence of old and new antibiotic-resistant bacterial strains in the last decades constitutes a substantial need for new classes of anti-bacterial agents.[2]

From the chemotherapeutic point of view, there are two sources of new chemical entities. The first is the extraordinary diversity provided by natural products. The second results from the design of new or the modification of synthetic transformations.

Chalcones or 1,3-diaryl-2-propen-1-ones, one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based food stuff have been recently subjects of their interesting pharmacological activities.[3] Chalcones are belong to the flavonoid family. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β,-unsaturated carbonyl system.

On the other hand, sulfur and/or nitrogen heterocycles that possess pharmaceutical activities widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of plant and animal cells. Penicillins containing a thiazole ring system (thiazolidine).[4] are also important naturally occurring products. Thiazoles and their derivatives are found to be associated with various biological activities such as antimicrobial[5], antituberculosis[6], and anti-inflammatory[7] activities. Previous studies have reported that flavones[8-9] derivatives (compound A & B, Fig. 1) or chalcone[10-11] derivatives (Compound E [Caffeic acid amides] & Compound F [Licochalcone], and Fig. 1) possessing antimicrobial activities. In the interest of the above suggestion and also continuous research on chalcone[12], we planned to design, synthesize a system that combines together two biolabile components which are flavones / chalcone and 2-amino thiazoles, to give a compact structure like the title compounds and highlighted their *in-vitro* antibacterial and antifungal activity.

2. EXPERIMENTAL PROTOCOLS

2.1. Materials and Methods

Starting materials were obtained from commercial sources and were used without further purification. Reaction progress was observed by thin layer chromatography using commercial silica gel plates (Merck Ltd., Mumbai). Melting points were determined in open capillary tubes on a Sonar melting point apparatus and are uncorrected. The infrared (IR) spectra were run as KBr disk on Jasco FTIR 4100 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined by Bruker 300 MHz FT- NMR spectrometer in appropriate deuterated solvents and are expressed in parts per million (δ, ppm) down feld from tetramethylsilane (internal standard). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Elemental analysis (C, H, and N) was undertaken with Elemental vario EL III Carlo Erba 1108 analyzer.

2.2.1 Synthesis of 2-amino thiazoles (Compound 3a-d)[13]

A mixture of acetophenone **(1)** (0.1mol), thiourea **(2)** (0.2 mol) and iodine (0.1 mol) was refluxed under electric water bath for 4-5 hrs at 70-80°C. Cool the content and extract with diethyl ether, yellow residue obtained. To the yellow residue add boiling distilled water and filter immediately while hot. Add strong ammonia solution drop wise to make the solution alkaline and product separated out, filter, collected, dried and recrystallized with ethanol to get pale yellow to brown products (Compound **3a-d**). Completion of the chemical reaction as indicated by TLC.

2.2.2. Synthesis of 1-(5-chloro-2-hydroxyphenyl)-3-phenylprop-2-en-1-one (6)[14]

i) Conventional method

A mixture of 1-(5-chloro-2-hydroxyphenyl)ethanone **(4)** (0.01 mol), benzaldehyde **(5)** (0.01 mol), sodium hydroxide (0.04 mol) and ethanol (20 mL) was stirred for 4 hrs at room temperature. After completion of reaction as indicated by TLC, the reaction mixture was poured on to crushed ice and neutralized with dilute HCl. The solid separated was filtered and recrystallized, yellowish product obtained.

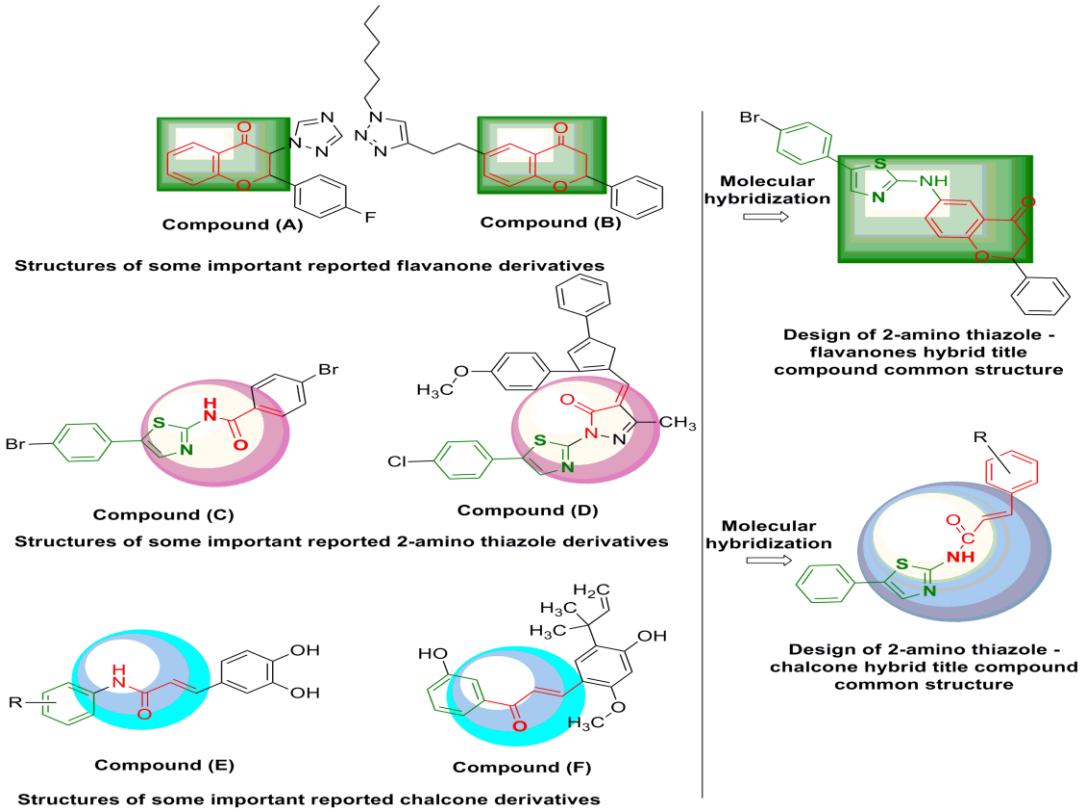


Fig 1: Design of molecular hybrids thiazole containing flavone / chalcone moieties.

ii) Microwave irradiation method

A mixture of 1-(5-chloro-2-hydroxyphenyl)ethanone **(4)** (0.001 mol), benzaldehyde **(5)** (0.001 mol), sodium hyoxide (0.004 mol) and ethanol (5 mL) was taken in a quartz tube and inserted into teflon vial with screw capped and subjected to microwave irradiation at 100 W (approximate : 70°- 85°C) for 5 min. After completion of reaction as indicated by TLC, the reaction mixture was poured on to crushed ice and neutralized with dilute hydrochloric acid. The solid separated was filtered and recrystallized, yellowish brown product obtained.

2.2.3. Synthesis of 6-chloro-2-phenyl-4H-chromen-4-one(7)[15]

i) Conventional method

Compound Chalcone **(6)** (1 m mol) and Iodine (0.1 m mol) in DMSO (20 mL) was refluxed for 30 min. Reaction mixture was poured on to crushed ice, filtered, washed with aqueous 20% sodium thiosulphate till the product become colorless. Product was filter, dried and recrystallized with ethanol.

ii) Microwave irradiation method

Compound Chalcone **(6)** (1 m mol) and Iodine (0.1 m mol) in DMSO (2 mL) was) was taken in a quartz tube and inserted into teflon vial with screw capped and subjected to microwave irradiation at 100 W (approximate : 70°- 85°C) for 3 min. Reaction mixture was poured on to crushed ice, filtered, washed with aqueous 20% sodium thiosulphate till the product become colorless. Product was filter, dried and recrystallized with ethanol.

2.2.4. Synthesis of 6-(4substituted thiazol-2-ylamino)-2-phenyl-4H-chromen-4-one (8a-d)

Equal mole mixture (0.1 mol) of 4-substituted thiazol-2-amine **(3a-d)**, with 6-chloro-2-phenyl-4H-chromen-4-one **(7)** stirred for 1- 2 hrs in the presence of base like pyridine in DMF. After completion of the reaction was checked by TLC was poured to ice cold water. The solid was separated, washed with water, dried and recrystallized.

2.2.5. Synthesis of N-(4-phenylthiazol-2-yl)acetamide[16] (Compound 9)

Compound **(3)** 2-amino thiazoles (0.0007 mole), acetyl chloride (0.0007 mole), triethylamine (0.001) and Dimethylformamide were stirred (magnetic stirrer) at room temperature for 1 hr. The mixture is then poured into crushed ice, the separated solid was filtered, washed with water, dried and recrystallized as yellow powder **(compound 9)**.

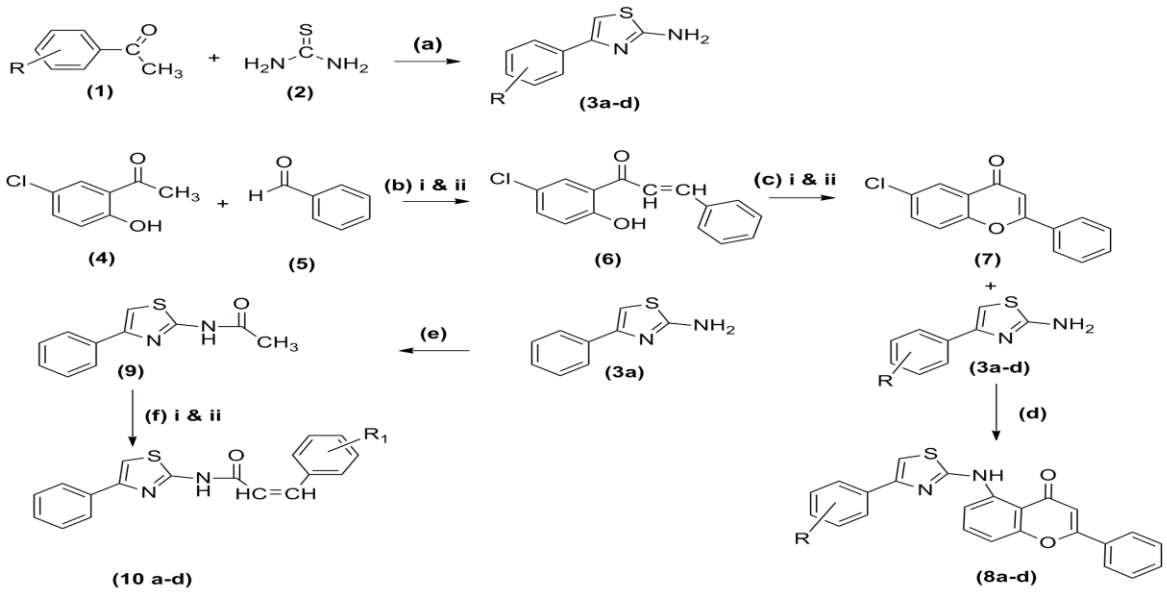
2.2.6. Synthesis of 3-(substituted phenyl)-N-(4-phenylthiazol-2-yl) acrylamide[14] (Compound 10a-d)

i) Conventional method

A mixture of N-(4-phenylthiazol-2-yl) acetamide **(9)** (0.01 mol), substituted benzaldehyde (0.01mol), sodium hydroxide (0.04 mol) and ethanol (20 mL) was stirred for 3-4 hrs at room temperature. After completion of reaction as indicated by TLC, the reaction mixture was poured on to crushed ice and neutralized with dilute HCl. The solid separated was filtered and recrystallized , colored product obtained **(10a-d)**.

ii) Microwave irradiation method

A mixture of N-(4-phenylthiazol-2-yl) acetamide **(9)** (0.001 mol), benzaldehyde (0.001 mol), sodium hydroxide (0.004 mol) and ethanol (5 mL) was taken in a quartz tube and inserted into Teflon vial with screw capped and subjected to microwave irradiation at 100 W (approximate : 70°- 85°C) for 4-5 min. After completion of reaction as indicated by TLC, the reaction mixture was poured on to crushed ice and neutralized with dilute hydrochloric acid. The solid separated was filtered and recrystallized, colored product obtained **(10a-d)**.



Where as: R (8a) H; (8b) 4-Cl; (8c) 4-OH; (8d)-CH_3
R_1 (10a) 4-NO_2; (10b) 4-OH; (10c) 4-Cl; (10d) 3,4-Cl

Scheme 1: Reagents and conditions

(a) I_2, 4-5 hrs refluxed, extract with ether, adjust PH with NH_3; **(b) i)** NaOH, C_2H_5OH, stirred 4 hrs; **ii)** NaOH, C_2H_5OH, MWI, 100W, 5 Min; **(c) i)** I_2, DMSO, refluxed 30 min, washed with $Na_2S_2O_3$; **ii)** I_2, DMSO, MWI, 100W, 5 Min washed with $Na_2S_2O_3$; **(d)** Pyridine in DMF, stirred 1-2 hrs; **(e)** Acetyl chloride, Triethyl amine, stirred 1 hr, neutralized with HCl; **(f) i)** NaOH, C_2H_5OH, stirred 3-4 hrs; **ii)** NaOH, C_2H_5OH, MWI, 100W, 5Min.

Fig 2: Synthetic scheme of novel hybrids thiazole containing flavone / chalcone moieties.

2.3. Antimicrobial evaluation

2.3.1. Determination of zone of inhibition

All newly synthesized compounds (**8a-d & 10a-d**) were screened for their preliminary antibacterial activity against four Gram-positive strains: *Bacillus lintus*, *Bacillus substilis*, *Micrococcus luteus*, and *Staphylococcus albus*; four Gram-negative strains: *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *S. paratyphi*,; and three fungal strains: *Aspergillus parasite*, *Aspergillus fumigates*, *Candida albicans*, and *Monococcus purpuram* by disc diffusion method.[17] A standard inoculum (1-2 × 10^7 cfu/mL 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile cotton swab was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140°C for 1 hrs. The sterile discs previously soaked in a known concentration (100 µg/mL) of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 hrs at 37 ± 1°C for bacteria and 72-96 h at 27 ± 1°C for fungi. After the incubation zone of inhibition was measured. The media used was nutrient agar medium and Sabouraud dextrose medium for antibacterial and antifungal activity, respectively. Ciprofloxacin (5 µg/disc) and Clotrimazole (5 µg/disc) were used as standard drugs for antibacterial and antifungal activity, respectively. Triplicate was maintained for all tested strains. Activity was determined by measuring the diameter of the zone showing complete inhibition. The average mean results of the antibacterial and antifungal studies are listed in Tables 3&5, respectively.

2.3.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration[17] (MIC) in µg/mL of the titled compounds was carried out by two-fold serial dilution method. The synthesized compounds (**8a-d & 10a-d**) were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth from 24 hrs old bacterial cultures on nutrient agar at 37 ± 1°C while fungal spores from 1 to 7 days old Sabouraud agar slant cultures were suspended in Sabouraud dextrose broth. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 104-105 cfu/mL. The final inoculums size was 105 cfu/mL for antibacterial assay and 1.1-1.5 cfu/mL × 102 cfu/mL for antifungal assay. Testing is performed at pH 7.4 ± 0.2 for bacteria and at a pH 5.6 for fungi. 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 mL of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six of such dilutions are obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The MICs were recorded through visual observations after 24 hrs (for bacteria) and 72-96 hrs (for fungi) of incubation. Ciprofloxacin was used as standard for bacterial studies and Clotrimazole was used as standard for fungal studies. The lowest concentration at which there was no visible growth was taken as MIC. The results of the MIC study are listed in Tables 4& 6.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic pathway for the synthesis of the targeted compounds is illustrated in **Scheme 1**. Compound 4-(4-substituted phenyl)thiazol-2-amine **(3a-d)** were prepared by using various acetophone with thiourea. The chalcone compound 1-(5-chloro-2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**6**) and titled chalcone compounds (**10a-d**) were synthesized [Claisen-Schmidt condensation] by using various aldehydes according to previously reported procedures with little modification. Intermediate compound 6-chloro-2-phenyl-4H-chromen-4-one (**7**) was prepared by cyclization of compound **(6)** with iodine in presence of DMSO by Microwave assisted techniques as well as conventional method. The titled compounds **(10 a-d)** and intermediate compound **(6 and 7)** were achieved by both microwave (ecofriendly) and conventional (Traditional) methods. The physicochemical data are described in **Table 1**. Microwave assisted techniques are found to be more effective in perspective of environment, reaction time, high yields, ease of work-up and isolation of products. More over microwave irradiation offers several advantages:[18] solvents are often expensive, toxic, difficult to remove in the case of aprotic dipolar solvents with high boiling point, and are environmentally polluting agents. Time and yield data of newly synthesized intermediate and titled compounds (**6, 7 & 10a-d**) by microwave and conventional methods were given in **Table 2**. Compound (**9**) was achieved by N-acylation (Schotten-Baumann method) of compound **3a** with acetyl chloride in presence of base readily converted to more stable and pure compound amide. Structure of the synthesized intermediate and titled compounds (**6, 7, 9, 8a-d & 10 a-d**) were established on the basis of physicochemical, elemental analysis and spectral data (IR, and ^{1}HNMR). The IR spectrum of compounds (**3a-d**) shows an absorption band at between 3251 to 3228 cm^{-1}, corresponding to the vibration of the NH_2 of 2-aminothiazole, a band at 1612 to 1618 cm^{-1}, characteristic of the C=N, while the compounds (**8a-d**) spectra showed the disappearance of the characteristic bands of the primary amine and the appearance of strong bands in the 3389 to 3391 cm^{-1} region, attributed to NH group stretching. Structures of key intermediates (**9**) showed that the disappearance of the characteristic bands of NH_2 group stretching. Compound **(7)** was cyclization of compound (**6**) was confirmed by the IR spectra which showed a single C=O band of flavone ring (1645 cm^{-1}). Proton assignments in ^{1}H-NMR spectra for compound (**3a-d**) showed signals at δ 6.78-6.57 (s, 1H, C4 thiazole ring), 6.50-6.26 (s, 2H, NH_2), while compounds (**8a-d**) showed the disappearance of the characteristic signals for

the primary amine group, and appearance of secondary amine signals at δ 3.62 – 3.46 (s, 1H, NH-). The key intermediate (**9**) showed the absence of the signals for the -NH_2 group, while the Methyl -CH_3 signal appeared at δ 3.52 ppm. Formation of chalcone (CO-CH=) (6 & 10a-d**)** confirmed doublet signals in the range 5.85-5.72 (NH) and 8.70-8.59 (Ar-CH=) δ ppm. Compounds **8c** &**10b**, showed the appearance of the characteristic signals for aromatic OH at δ 8.68 &8.48 ppm, respectively. Methyl (-CH_3) containing titled compound **8d** showed the appearance of the characteristic signals at δ 3.20 ppm. Compound **9** containing CH_3 group signal appeared at δ 3.52 ppm. Further, the formation of title compounds was confirmed by recording their mass spectrums which were in full agreement with their molecular weights and the results of elemental analysis (carbon, hydrogen and nitrogen) were ± 0.4 % of the theoretical values. In conclusion, we have synthesized chalcone derivatives using microwave assisted techniques and are more convenient, environmentally safe as they require less volume of solvent, short reaction span and better yields as compared to conventional techniques.

3.1.1. Compound 4-phenylthiazol-2-amine (3a)

IR (KBr, cm^{-1}): 3241 (thiazole -NH_2 group), 3021 (Ar-CH stretching), 1617 (C=N stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 7.48–8.26 (m, 5 H, Ar-H), 6.75 (s, 1H, C4 of thiazole ring), 6.50 (s, 2H, -NH_2); Exact Mass: m/z: 176.04 [M]+; Anal. Calculated: C, 61.34; H, 4.58; N, 15.90; S, 18.19; Found: C, 60.95; H, 4.47; N, 15.43.

Table-1: Physicochemical parameter of synthesized compounds (8a-d &10a-d).

S. No	Code	Molecular formula	M. Wt (g)	MP range (°C)	*R_f Value	Refractivity index	Log-p	C Log-P	Polarizability
1	6	$C_{15}H_{11}ClO_2$	258.04	102-105	0.85	73.66	4.84	4.79	27.52
2	7	$C_{15}H_9ClO_2$	256.03	106-108	0.91	71.78	3.57	4.19	27.12
3	8a	$C_{24}H_{16}N_2O_2S$	396.46	162-167	0.78	116.11	5.62	5.98	45.38
4	8b	$C_{24}H_{15}N_2O_2S$	430.90	172-178	0.82	120.91	6.22	6.70	47.21
5	8c	$C_{24}H_{16}N_2O_3S$	412.46	160-166	0.73	118.09	5.31	5.52	45.95
6	8d	$C_{25}H_{18}N_2O_2S$	410.48	159-165	0.72	121.15	6.13	6.48	47.14
7	10a	$C_{18}H_{13}N_3O_3S$	351.07	98-103	0.82	97.10	4.89	4.74	37.06
8	10b	$C_{18}H_{14}N_2O_2S$	322.08	95-101	0.92	92.76	4.64	4.33	35.82
9	10c	$C_{18}H_{13}ClN_2OS$	340.04	96-105	0.89	95.59	5.55	5.71	37.07
10	10d	$C_{18}H_{12}Cl_2N_2OS$	374.00	99-106	0.81	100.39	6.15	6.30	38.96

*Benzene: Methanol: Ethyl acetate (7:2:1)

Table 2: Percentage yield of chalcone derivatives.

S.No	Code	Percentage Yield of chalcone derivatives				
		Conventional method		Microwave irradiation method		
		Time (hrs)	Percentage Yield	Time (min)	Power (W)	Percentage Yield
1	6	4.00	71	5	100	87
2	7	0.50	65	3	100	82
3	10a	3.40	52	5	100	68
4	10b	3.30	60	5	100	70
5	10c	3.40	58	5	100	67
6	10d	4.00	61	5	100	69

3.1.2. Compound 4-(4-chlorophenyl)thiazol-2-amine (3b)

IR (KBr, cm^{-1}): 3239 (thiazole -NH_2 group), 3026 (Ar-CH stretching), 1615 (C=N stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 7.24–8.16 (m, 4 H, Ar-H), 6.78 (s, 1H, C4 of thiazole ring), 6.40 (s, 2H, -NH_2);Chemical Formula: $C_9H_7ClN_2S$; Exact Mass: m/z: 210.00 [M]+; Anal. Calculated: C, 51.31; H, 3.35; Cl, 16.83; N, 13.30; S, 15.22; Found: C, 51.05; H, 3.47; N, 13.13.

3.1.3. Compound 4-(2-aminothiazol-4-yl)phenol (3c)

IR (KBr, cm^{-1}): 3251 (thiazole -NH_2 group), 3112 (Ar-CH stretching), 1612 (C=N stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 7.16–8.24 (m, 4 H, Ar-H), 6.69 (s, 1H, C4 of thiazole ring), 6.38 (s, 2H, -NH_2), 5.50 (s, 1H, Ar-OH);Chemical Formula: $C_9H_8N_2OS$; Exact Mass: m/z: 192.04 [M]+; Anal. Calculated: C, 56.23; H, 4.19; N, 14.57; O, 8.32; S, 16.68; Found: C, 55.98; H, 3.89; N, 13.43.

3.1.4. Compound 4-(p-tolyl)thiazol-2-amine (3d)

IR (KBr, cm^{-1}): 3228 (thiazole -NH_2 group), 3198 (Ar-CH stretching), 1618 (C=N stret); ^{1}H NMR (DMSO-d_6) δ ppm: 7.21–8.29 (m, 4 H, Ar-H), 6.57 (s, 1H, C4 of thiazole ring), 6.26 (s, 2H, -NH_2), 3.95 (s, 3H, Ar-CH_3);Chemical Formula: $C_{10}H_{10}N_2S$; Exact Mass: m/z: 190.06 [M]+; Anal. Calculated: C, 63.13; H, 5.30; N, 14.72; S, 16.85; Found: C, 62.98; H, 5.19; N, 14.29.

3.1.5. Compound 1-(5-chloro-2-hydroxyphenyl)-3-phenylprop-2-en-1-one (6)

IR (KBr, cm^{-1}): 3198 (Ar-CH stretching), 1618 (-NH-CO- bending), 882 (Ar-CH=CH-), 841 (Ar C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 8.79 (s, 1H, Ar-OH), 8. 57 (d, 1H, Ar-CH=), 6.87–7.98 (m, 7 H, Ar-H), 6.42 (s, 1H, C4 of thiazole ring), 6.15 (s, 2H, -NH_2), 5.85 (d, 1H, -CO-CH=); Chemical Formula:$C_{15}H_{11}ClO_2$; Exact Mass: m/z: 258.04 [M]+; Anal. Calculated: C, 69.64; H, 4.29; Cl, 13.70; O, 12.37; Found: C, 69.21; H, 4.19; N, 13.35.

3.1.6. Compound 6-chloro-2-phenyl-4H-chromen-4-one (7)

IR (KBr, cm^{-1}): 3230(Ar-CH stretching), 1645.55, (C=O str., flavone ring), 839 (Ar C-Cl stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 6.87–7.98 (m, 7 H, Ar-H), 5.85 (d, 1H, -CO-CH=); Chemical Formula:$C_{15}H_9ClO_2$; Exact Mass: m/z: 256.03 [M]+; Anal. Calculated: C, 70.19; H, 3.53; Cl, 13.81; O, 12.47; Found: C, 69.98; H, 3.19.

3.1.7. Compound 2-phenyl-6-((4-phenylthiazol-2-yl)methyl)-4H-chromen-4-one(8a)

IR (KBr, cm^{-1}): 3389.24 (NH str.), 1640.55, (C=O str., flavone ring), 1576.62 (C=N str.), 768.35 (CH=CH str., aromatic); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.13-7.96 (m, 11 H, Ar-H), 6.65-6.81 (m 4H, flavone ring), 3.46 (s, 1H, NH of secondary amine); Exact Mass: m/z: 396.06 [M]+; Anal. Calculated for $C_{24}H_{16}N_2O_2S$: C, 72.71; H, 4.07; N, 7.07; O, 8.07; S, 8.09: Found: C, 72.14; H, 3.52; N, 6.95.

3.1.8. Compound 6-((4-(4-chlorophenyl) thiazol-2-yl)methyl)-2-phenyl-4H-chromen-4-one(8b)

IR (KBr, cm-1): 3393.12 (NH str.), 16390.24, (C=O str., flavone ring), 1579.58 (C=N str.), 842 (Ar C-Cl stretching), 771.25 (CH=CH str., aromatic); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.21-7.95 (m, 10 H, Ar-H), 6.61-6.90 (m 4H, flavone ring), 3.62 (s, 1H, NH of secondary amine); Exact Mass: m/z: 430.05 [M]+; Anal. Calculated for $C_{24}H_{15}ClN_2O_2S$: C, 66.90; H, 3.51; Cl, 8.23; N, 6.50; O, 7.43; S, 7.44: Found: C, 66.54; H, 3.222; N, 6.34.

3.1.9. Compound 6-((4-(4-hydroxyphenyl)thiazol-2-yl)methyl)-2-phenyl-4H-chromen-4-one(8c)

IR (KBr, cm-1): 3391.34 (NH str.), 16387.56, (C=O str., flavone ring), 1581.56 (C=N str.), 768.35 (CH=CH str., aromatic); ^{1}H-NMR (DMSO-d_6) δ ppm: 8.68 (s, 1H, Ar-OH), 7.18-7.86 (m, 10 H, Ar-H), 6.68-6.85 (m 4H, flavone ring), 3.50 (s, 1H, NH of secondary amine); Exact Mass: m/z: 412.09 [M]+; Anal. Calculated for $C_{24}H_{16}N_2O_3S$: C, 69.89; H, 3.91; N, 6.79; O, 11.64; S, 7.77: Found: C, 69.74; H, 3.62; N, 6.40.

3.1.10. Compound 2-phenyl-6-((4-(p-tolyl)thiazol-2-yl)methyl)-4H-chromen-4-one(8d)

IR (KBr, cm-1): 3389.85 (NH str.), 16390.13, (C=O str., flavone ring), 1583.32 (C=N str.), 768.35 (CH=CH str., aromatic); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.12-7.92(m, 10H, Ar-H), 6.52-6.89 (m 4H, flavone ring), 3.56 (s, 1H, NH of secondary amine), 3.20 (s, 3H, -CH_3); Exact Mass: m/z: 410.11 [M]+; Anal. Calculated for $C_{25}H_{18}N_2O_2S$: C, 73.15; H, 4.42; N, 6.82; O, 7.80; S, 7.81: Found: C, 72.94; H, 4.12; N, 6.39.

3.1.11. Compound N-(4-phenylthiazol-2-yl)acetamide (9)

IR (KBr, cm^{-1}): 3336 (thiazole N-H group), 3028 (Ar-CH stretching), 1696 (C=O stretching), 1619 (C=N stretching); ^{1}H NMR (DMSO-d_6) δ ppm: 3.52 (S, 3H, CH_3), 7.08–8.12 (m, 5 H, Ar-H), 6.69 (s, 1H, C4 of thiazole ring), 10.20 (s, 1H, -NH). Exact Mass: m/z: 218.05 [M]+; Anal. Calculated: C, 60.53; H, 4.62; N, 12.83; O, 7.33; S, 14.69; Found: C, 60.15; H, 4.57; N, 12.43.

3.1.12. Compound 3-(4-nitrophenyl)-N-(4-phenylthiazol-2-yl)acrylamide (10a)

IR (KBr, cm^{-1}): 3237 (NH Stretch), 2689 (Ar.CH Stretch), 1724 (C=O Stretch), 1619 (C=O); 1550 (N=O stretching); 1509 (Ar C=C ring stretching), 1480 (-$NHCOCH_2$-). ^{1}H NMR (DMSO-d_6) δ ppm: 5.72 (d, 1H, -CO-CH=), 6.08–8.33 (m, 09 H, Ar-H), 8. 62 (d, 1H, Ar-CH=), 9.24 (s, 1H, C4 of thiazole ring), 10.16 (s, 1H, Ar-NH). Exact Mass: m/z: 351.07 [M]+; Anal. Calculated for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96; O, 13.66; S, 9.13: Found: C, 61.12; H, 3.42; N, 11.28.

3.1.13. Compound 3-(4-hydroxyphenyl)-N-(4-phenylthiazol-2-yl)acrylamide (10b)

IR (KBr, cm^{-1}): 3532 (thiazole N-H group); 2821 (Ar-CH stretching); 1710 (C=O stretching); 1613 (C=N stretching); 1515 (Ar C=C stretching). ^{1}H NMR (DMSO-d_6) δ ppm: 5.85 (d, 1H, -CO-CH=), 7.18–8.40 (m, 10 H, Ar-H), 8.67 (d, 1H, Ar-CH=), 9.86 (s, 1H, C4 of thiazole ring), 10.95 (s, 1H, Ar-NH). Exact Mass: m/z: 322.08 [M]+; Anal. Calculated for $C_{18}H_{14}N_2O_2S$: C, 67.06; H, 4.38; N, 8.69; O, 9.93; S, 9.95: Found: C, 66.54; H, 4.12; N, 8.29.

3.1.14. Compound 3-(4-chlorophenyl)-N-(4-phenylthiazol-2-yl)acrylamide (10c)

IR (KBr, cm^{-1}): 3471 (thiazole N-H group); 2982 (Ar-CH stretching); 1704 (C=O stretching); 1611 (C=N stretching), 1513 (Ar C=C stretching); 831 (Ar C-Cl stretching). ^{1}H NMR (DMSO-d_6) δ ppm: 5.79 (d, 1H, -CO-CH=), 7.06–8.83 (m, 10 H, Ar-H), 8.70 (d, 1H, Ar-CH=), 9.94 (s, 1H, C4 of thiazole ring), 11. 68 (s, 1H, Ar-NH). Exact Mass: m/z: 340.04 [M]+; Anal. Calculated for $C_{18}H_{13}ClN_2OS$: C, 63.43; H, 3.84; Cl, 10.40; N, 8.22; O, 4.69; S, 9.41: Found: C, 63.13; H, 3.52; N, 8.12.

3.1.15. Compound 3-(3,4-dichlorophenyl)-N-(4-phenylthiazol-2 yl)acrylamide (10d)

IR (KBr, cm^{-1}): 3434 (thiazole N-H group); 2426 (Ar-CH stretching); 1644 (C=O stretching); 1597 (C=N stretching) 1503 (Ar C=C stretching); 737 (Ar C-Cl stretching). ^{1}H NMR (DMSO-d_6) δ ppm: 5.75(d, 1H, -CO-CH=), 6.03–8.83 (m, 10 H, Ar-H), 8.59 (d, 1H, Ar-CH=), 9.90 (s, 1H, C4 of thiazole ring), 11.41 (s, 1H, Ar-

NH). Exact Mass: m/z: 374.00 [M]+; Anal. Calculated for $C_{18}H_{12}Cl_2N_2OS$: C, 57.61; H, 3.22; Cl, 18.89; N, 7.46; O, 4.26; S, 8.5: Found: C, 57.23; H, 3.09; N, 7.82.

3.2. Biology

All the synthesized compounds **6, 7, 8a-d & 10 a-d** (100µg/disc) were screened for their primary antimicrobial activity against four gram-positive, four gram-negative bacterial strains and four fungal strains by disc diffusion method. The compounds, which exhibited zone of inhibition in the primary screening, were evaluated for MIC (100 -1.56-µg/mL) by two fold serial dilution method in triplicates. The mean average values of antimicrobial results are presented in Tables 4 and 6.

Table-3: *Invitro* anti-bacterial activity of the synthesized compounds by discs diffusion method (8a-d &10a-d)

		Zone of Inhibition (in Mm)																						
		6	7		8a	8b	8c	8d	10a	10b	10c	10d	STD	Solvent	6	7		8a	8b	8c	8d	10a	10b	10c
		Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	DMSO
1	*E. coli*	21	54	-	-	-	-	-	-	-	-	-	-	30	81	20	51	27	69	35	90	39	100	-
2	*K. pneumoniae*	20	54	-	-	21	57	15	41	12	32	12	32	28	75	21	56	26	70	27	72	37	100	-
3	*P. aeroginosa*	20	50	12	30	19	48	12	30	11	28	10	25	26	65	22	55	29	72	29	73	40	100	-
4	*S. paratyphi*	20	51	10	26	-	-	11	28	-	-	-	-	30	76	20	51	28	71	29	74	39	100	-
5	*B. lintus*	14	37	12	32	20	53	13	34	11	30	12	32	28	73	20	53	29	76	33	87	38	100	-
6	*S. albus*	15	41	11	30	13	35	11	30	11	30	-	-	30	81	21	56	28	75	26	70	37	100	-
7	*B. subtilis*	14	35	13	33	12	30	11	28	11	28	-	-	27	67	25	62	26	65	29	73	40	100	-
8	*M. luteus*	16	41	15	38	20	51	16	41	12	31	13	33	31	79	20	51	29	74	36	92	39	100	-

(-) indicate - No zone of inhibition; STD- Ciprofloxacin

Table-4: *Invitro* anti-bacterial activity of the synthesized compounds (8a-d & 10a-d) by two-fold serial dilution method

S. No	Microorganisms	Minimum Inhibitory Concentration(MIC in µg/mL)											
		6	**7**	**8a**	**8b**	**8c**	**8d**	**10a**	**10b**	**10c**	**10d**	**STD**	**Solvent**
1	*E. coli*	25	NT	NT	NT	NT	NT	6.25	25.00	12.5	12.5	<1.5	-
2	*K. pneumoniae*	12.5	NT	25	25	25	25	6.25	50.00	12.5	12.5	<1.5	-
3	*P.aeroginosa*	12.5	12.5	12.5	25	25	25	25.00	50.00	12.5	12.5	<1.5	-
4	*S. paratyphi*	12.5	25	NT	25	NT	NT	25.00	50.00	50.00	50.00	<1.5	-
5	*B. lintus*	12.5	25	12.5	12.5	12.5	12.5	12.5	50.00	12.5	25.00	<1.5	-
6	*S. albus*	12.5	25	25	25	25	NT	6.25	50.00	6.25	6.25	<1.5	-
7	*B. subtilis*	12.5	25	25	25	25	NT	6.25	25.00	6.25	6.25	<1.5	-
8	*M. Luteus*	12.5	25	25	25	50	25	25.00	50.00	12.5	12.5	<1.5	-

NT- Not Tested; STD- Ciprofloxacin; Solvent- DMSO

Table-5: *Invitro* anti-fungal activity of the synthesized compounds (8a-d &10a-d)by discs diffusion method

S.NO	Micro organisms	Zone of Inhibition (in Mm)																					
		6		7		8a		8b		8c		8d		10a		10b		10c		10d		STD		Solvent
		Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	Mm	%	DMSO
1	*C. albicans*	15	47	20	63	33	103	14	44	-	-	-	-	12	43	14	44	25	65	11	34	32	100	-
2	*M. purpuram*	13	48	21	78	25	93	12	44	11	41	11	41	13	44	12	44	11	41	11	41	27	100	-
3	*A. parasite*	13	47	15	54	31	79	10	36	12	43	-	-	9	33	10	36	12	43	11	39	28	100	-
4	*A. fumigates*	14	58	18	75	20	83	12	50	-	-	12	50	12	50	6	25	7	29	12	50	24	100	-

(-) indicate - No zone of inhibition; STD-Clotrimazole

Table -6: *Invitro* anti-fungal activity of synthesized compounds (8a-d & 10a-d) by two-fold serial dilution method

S. No	Microorganisms	Minimum Inhibitory Concentration(MIC in µg/mL)											
		6	7	8a	8b	8c	8d	10a	10b	10c	10d	STD	Solvent
1	*C. albicans*	25	12.5	12.5	12.5	NT	NT	25	25	12.5	12.5	<1.5	-
2	*M.purpuram*	25	25	6.25	12.5	12.5	50.0	25	25	12.5	12.5	<1.5	-
3	*A. parasite*	25	12.5	6.25	12.5	12.5	NT	25	25	12.5	12.5	<1.5	-
4	*A. fumigates*	25	6.5	6.25	12.5	NT	12.5	12.5	25	6.25	6.25	<1.5	-

NT - Not Tested; STD-Clotrimazole; Solvent- DMSO

The results of antibacterial screening showed that chalcone containing titled compounds (**10a-d**) demonstrated significant activity against both Gram-positive and Gram-negative bacterial strains as well as fungal strains compared to the reference Ciprofloxacin (for bacteria) and Clotrimazole (for fungi). Among the synthesized compounds, chalcone bearing thiazole derivatives (**10a-d**) showed better antimicrobial activity (> 50%) compare with flavone bearing thiazole derivatives (**8a-d**). Intermediate compound (**6**) demonstrate good activity against all tested strains compare that of flavone derivative (**7**). In chalcone bearing thiazole derivatives (**10a-d**) having electron donating groups (4-OH) on the phenyl ring were reduce its antibacterial properties. In case of *E.Coli, B. lintus and M.Luteus*, the compounds **10a**, and **10d**, displayed significant activity with % of inhibition between 79-85. On the other hand, thiazol-2-amino-flavone derivatives (**8a-d**) showed % of inhibition between 25-58 . If the compounds having refractivity index less than 100 and polarizability between 35- 39 showed better antimicrobial activity. In case of antifungal activities against *C. albicans*, the compound **8a**, displayed equipotent antifungal activity as that of standard Clotrimazole with % of inhibition 103 and MIC at 12.5 µg/mL. Compound **8a** showed significant activity against all other three tested fungal strains with % of inhibition range between 79-93 and MIC at 6.25 µg/mL. Compounds **8c** and **8d** having electron donating groups on the phenyl ring were not showed antifungal activity as compounds (**8a & 8b**) with un-substituted phenyl / electron withdrawing group in the phenyl ring structure. On the other hand, compounds **10a-d** series demonstrate mild antifungal activity with % of inhibition < 50 and MIC range between 12.5 -25 µg/mL.

3.2.1. Structure activity relationship

From the results of the *in-vitro* antimicrobial activity of the synthesized intermediate, and titled compounds **6,7, 8a-d & 10a-d** the following structure activity relationship (SAR) can be derived.

a) Among the synthesized compounds, Compound with thiazol-2-amino-chalcone derivatives (**10a-d**) showed better *in-vitro* antibacterial activity compare to thiazol-2-amino-flavones derivatives (**8a-d**).

b) In thiazol-2-amino-chalcone derivatives (**10a-d**), the deactivating substituent's compounds (**10d**) on the phenyl ring, the highest antibacterial activity was obtained with highest lipophilicity, highest polarizability and withdrawing power .

c) In thiazol-2-amino-flavanone derivatives (**8a-d**), Presence of electron withdrawing group on the phenyl ring or unsubstituted phenyl ring is responsible for better antifungal activity against.

d) Intermediate compound (**6**) better antimicrobial activity compared to that of intermediate cyclized flavones derivative **(7)**

4. CONCLUSION

In conclusion, a series of four thiazol-2-amino-chalcone derivatives (**10a-d**) and four Schiff thiazol-2-amino-flavanone derivatives (**8a-d**) were synthesized and their antibacterial activities against four Gram-positive, four Gram-negative strains and antifungal activities against three fungal strains were evaluated. According to the primary antimicrobial screening, the compound (**8a)**, displayed equipotent antifungal activity against *C. albicans* as that of standard Clotrimazole with % of inhibition 103 and MIC at 12.5 µg/mL. Among the synthesized compounds, Compound with thiazol-2-amino-chalcone derivatives (**10d)** showed the significant antibacterial activity. We concluded from our investigations that **8a**, **10c** and **10d** may be considered promising for the development of new antimicrobial agents.

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