Research Article

Design, Synthesis and in vitro antimicrobial screening of some novel N-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives

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Abstract

A new series of twelve N-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives (5a-l) containing 2,4-dichlorophenyl moiety were synthesized via the condensation reaction between 1-(2,4-dichlorophenyl)thiourea (3) with different phenacyl halides (4). The chemical structures of novel derivatives were established on the basis of IR, 1H-NMR, 13C-NMR, mass spectroscopy and elemental analyses. The newly synthesized compounds were screened for their in vitro antibacterial activity against Gram positive bacteria viz., Staphylococcus aureus, Bacillus subtilis and Gram-negative bacteria namely Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumonia. The antifungal properties were studied by evaluating against fungal strains namely Aspergillus niger, Aspergillus flavus, Candida albicans and Neurospora crassa. Among screened, title compounds 5a, 5b, 5e 5g and 5i exhibited potential activity against all the bacterial pathogens. Compound 5a demonstrated exceptional microbial inhibition property.

Keywords: anti-bacterial activity, phenacyl halide, candida albican, aspergillus niger and Gram positive.

1. Introduction

In recent years, much attention has been paid by the researchers to cure infectious diseases. Although there are several antibiotic drugs are available in the market, the infectious microbes develop resistance to the existing drugs. There is clearly a critical need for development of selective, less toxic and potent antibacterial agents. Thiazole derivatives occupy prominent position in the field of agriculture and medicine due to their broad spectrum biological properties. Various thiazole derivatives have been reported to possesses potential biological activities and used in the treatment of allergies[1], inflammation[2], hypertension[3], bacterial infection[4], schizophrenia[5], hypnotics[6], HIV infections[7] and thrombosis[8]. Sulfathiazole (antimicrobial drug), bleomycin and tiazofurin (antineoplastic drug) are some of the thiazole containing drugs. It has been found that 2-amino-4-phenylthiazole derivatives have shown anaesthetic activity[9]. A large number of thiazole- containing compounds are reported as herbicidal, fungicidal, antitubercular, antianaphylactic, antibiotic, antiviral antiarthritic, antianalgesic and psychotropic agents[10-22]. Recently, aminothiazoles were also synthesized by using various catalysts such as iodine[23], silica chloride[24], 1,3-dinbutylimidazolium tetrafluoroborate [25] ammonium 12-molybdopho- sphate and cyclodextrin.[26,27].

In view of above mentioned observations and stimulated by the biological significance of thiazole derivatives, we have designed the synthesis of ***N***-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** by the refluxing 1-(2,4-dichlorophenyl)thiourea **(3)** with different phenacyl halides **(4)** in ethanol for 16 hours at 80^0C **(Scheme 2).** All the novel compounds were obtained in good yield. The newly synthesized derivatives were screened for their *in vitro* antimicrobial activity studies against both Gram positive and Gram negative bacteria. Among tested, compound **5a, 5f, 5g** and **5i** showed potential activity towards most of the pathogens.

2. Pharmacology

The newly synthesized *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** were evaluated for their antibacterial and antifungal properties.

2.1. Anti-bacterial activity

After structural confirmation by spectroscopic study, the newly synthesized *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** were tested for their *in vitro* antibacterial activity studies against Two Gram-positive bacteria viz., *Staphylococcus aureus* MTCC-7443, *Bacillus subtilis* MTCC-441 and four Gram-negative bacteria namely *Escherichia coli* MTCC-725*, Pseudomonas aeruginosa* MTCC-424*, Proteus vulgaris* MTCC-426 and *Klebsiella pneumoniae* MTCC-139 by Disc Diffusion Method (ZOI Test) [28] according to well established procedure in the literature. The microorganisms used for antibacterial assay were recommended

by the National Committee for Clinical Laboratories Standards (NCCLS) and procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. **Ampicillin**, an antibiotic drug at a dose of 10 µg/disc was used as a reference standard. The bacterial strains were inoculated on Mueller-Hinton Agar (MHA) and incubated for 24 h at 37°C, then suspended in saline solution 0.85% NaCl and adjusted to yield approximately $1.0x10^7$-$1.0x10^8$ cfu/mL by using spectrophotometer (25% transmittance at 530 nm) as per the guidelines given in Indian Pharmacopoeia (2007)[29].

Sterile empty discs (6mm diameter) were purchased from Himedia Company, Mumbai, 50 mg of compound powder was dissolved in 5 ml of DMSO (Dimethyl Sulphoxide), from the stock solution 100µl of respective DMSO extract was loaded on the disc individually and aseptically, after drying they were used for screening the antimicrobial assay. The test compounds were prepared with different concentrations using *N, N* dimethyl formamide. DMSO extracts were dissolved in 5 ml of their respective extraction solvent and antimicrobial activity was determined by the disc diffusion method (Vardarunler *et al*, 2003)[30]. Sterile discs (6.0 mm, Commercial disc, Hi Media, Mumbai) were saturated with 100 µl of the test solution, dried under laminar air flow and placed on the Mueller-Hinton Agar (MHA) plate, which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 37°C, for a period of 18-24 h. Discs treated with 100 µl Dimethyl sulphoxide was used as negative controls and Ampicillin discs (10 µg) were used as positive controls. The extracts and supernatants containing antibacterial components produced distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of antibacterial activity.

2.2. Antifungal activity

All the newly synthesized derivatives **(5a-l)** were screened for their antifungal activity against *Aspergillus niger, Aspergillus flavus, Candida albicans and Neurospora crassa* by disc fusion method. The fungal strains were inoculated on Potato Dextrose Agar (PDA) and inoculated for 48 hrs at 30° C, then suspended in saline solution 0.85% NaCl and adjusted to yield approximately $1.0x10^7$-$1.0x10^8$ cfu/mL by using spectrophotometer (25% transmittance at 530 nm) as per the guidelines given in Indian Pharmacopoeia (2007) [23].

DMSO extracts were dissolved in 5 mL of their respective extraction solvent and antimicrobial activity was determined by the disc diffusion method (Vardarunler *et al.*, 2003) [30]. Sterile discs (6.0 mm, Commercial disc, Hi Media, Mumbai) were saturated with 100 µl of the test solution, dried under laminar air flow and placed on Potato Dextrose Agar (PDA) plate for, which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 30°C, for 48 h. Discs treated with 100 µl Dimethyl sulphoxide was used as negative controls and **nystatin** disc (10 µg) used as positive control. The extracts and supernatants containing antifungal components produced distinct, clear, circular zones of inhibition around the disc and the diameters of clear zones were determined and minimum inhibitory concentrations (MICs) were determined at the lowest concentration that inhibited visible fungal growth.

3. Results and Discussion

3.1 Chemistry

The starting material, 1-(2,4-dichlorophenyl)thiourea **(3)** was prepared in the laboratory by refluxing equimolar quantities of 2,4-dichloroaniline **(1)** and ammonium thiocyanate **(2)** for 8 hours in ethanol as a solvent. A white solid product obtained which is recrystallized using hot ethanol **Scheme 1.**

The target compounds, *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** was synthesized by the refluxing 1-(2,4-dichlorophenyl)thiourea **(3)** with different phenacyl halides **(4)** for 16 hours at 80^0C (**Scheme II).** The novel N-substituted-(2,4-dichloro- phenyl)-1,3-thiazol-2-amines obtained in good yield and structures of all the synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, LC-Mass spectra and Elemental analyses .

3.2. Pharmacological screening

The antibacterial activity results of novel *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** indicated that all the synthesized novel compounds possess little or more activity on one or the other bacterial species. The result revealed that compound **5a, 5g** and **5i** exhibited excellent activity against all the six bacterial species. It is interpreted that, the outstanding activity of these derivatives is due the presence of 4-Br, 2,4-Cl_2 and 4-F pharmacological active moieties in the novel compounds. Compound **5b and 5f** showed a moderate bacterial inhibition against all the strains which were used in the study. The remaining compounds also displayed a comparable inhibition activity with the reference standard drug, **Ampicillin**. The results of the

study indicated that compound **5a, 5g and 5i** exhibited potential activity against all the six bacterial species. Hence they can be considered as broad spectrum antibacterial agents and antibacterial drug candidates for further studies in this line. Interestingly, all the bacterial species were found to be sensitive for these compounds. Compound **5k** exhibited relatively good activity against Gram-positive bacteria namely *Staphylococcus aureus* MTCC-7443, *Bacillus subtilis* MTCC-441 and Gram-negative bacteria namely *Escherichia coli* MTCC-725, *Pseudomonas aeruginosa* MTCC-424, MTCC-426 and *Klebsiella pneumoniae* MTCC-139. The results are tabulated in **table 1** and schematically shown in **figure 1.**

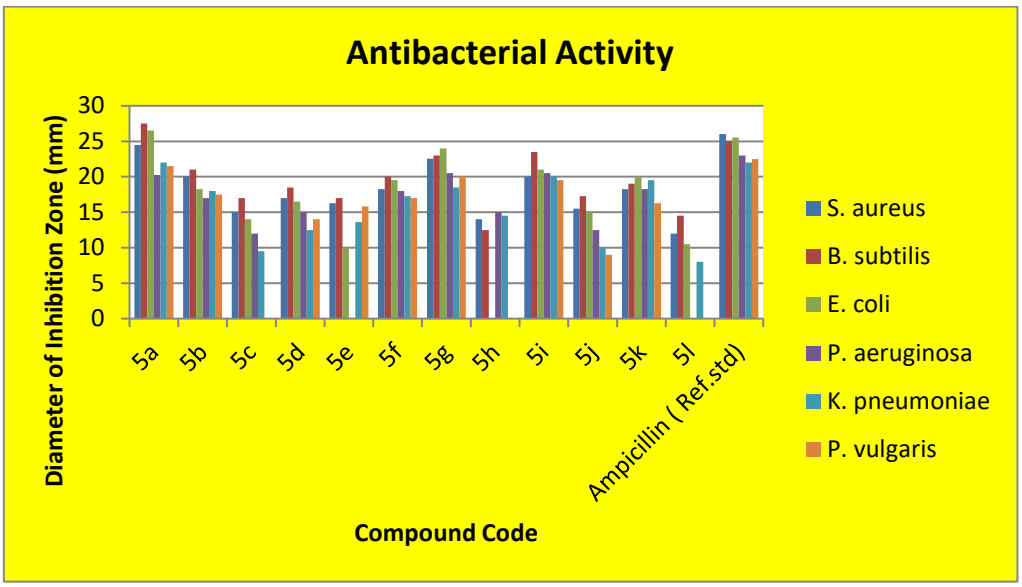


Figure 2: Antifungal Activity (Zone of Inhibition) of *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amines(5a-l) and Nystatin (Ref. standard).

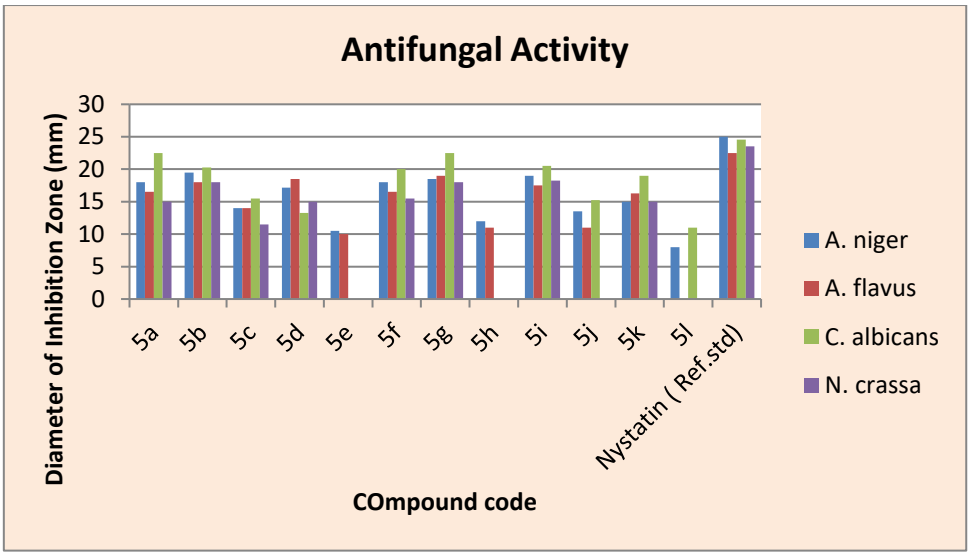


Table 1: Characterization data of novel *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amines (5a-l).

Sample	R^1	R	MF (MW)	m.p. (^oC) (Yield %)	% Composition found (Calculated) C	H	N
5a	-H	napthyl	$C_{19}H_{12}Cl_2N_2S$ (371.28)	111-113 (79)	61.46 (61.23)	3.26 (3.98)	7.55 (7.89)

5b	-H	4-Br-Ph	$C_{15}H_9BrCl_2N_2S$ (400.12)	95-97 (74)	45.03 (45.65)	2.27 (2.54)	7.00 (7.73)
5c	$-CH_3$	-Ph	$C_{17}H_{14}Cl_2N_2S$ (349.27)	72-76 (72)	58.46 (58.69)	4.04 (4.56)	8.02 (8.74)
5d	$-CH_3$	4-Br-Ph	$C_{16}H_{11}BrCl_2N_2S$ (414.14)	88-90 (68)	46.40 (46.52)	2.68 (2.71)	6.76 (6.31)
5e	-H	3,4-Cl_2-Ph	$C_{15}H_8Cl_4N_2S$ (390.11)	78-80 (81)	46.18 (46.89)	2.07 (2.83)	7.18 (7.41)
5f	-H	4,4^1-biPh	$C_{21}H_{14}Cl_2N_2S$ (397.32)	158-160 (72)	63.48 (63.59)	3.55 (3.45)	7.05 (7.63)
5g	-H	2,4-Cl_2-Ph	$C_{15}H_8Cl_4N_2S$ (390.11)	132-134 (76)	46.18 (46.01)	2.07 (2.52)	7.18 (7.23)
5h	-H	-SO_2CH_3-Ph	$C_{16}H_{12}Cl_2O_2N_2S_2$ (399.31)	116-120 (73)	48.13 (48.51)	3.03 (3.40)	7.02 (7.09)
5i	-H	4-F-Ph	$C_{15}H_9Cl_2FN_2S$ (339.21)	112-114 (80)	53.11 (53.60)	2.67 (2.60)	8.26 (8.21)
5j	$-CH_3$	$-CH_3$	$C_{11}H_{10}Cl_2N_2S$ (273.11)	102-104 (71)	48.36 (48.41)	3.69 (3.23)	10.25 (10.28)
5k	-H	$-CF_3$	$C_{10}H_5Cl_2F_3N_2S$ (313.12)	108-110 (73)	38.36 (38.43)	1.61 (1.59)	8.95 (8.90)
5l	-Cl	-Ph	$C_{15}H_9Cl_3N_2S$ (355.66)	68-70 (75)	50.65 (50.44)	2.55 (2.50)	7.88 (7.81)

Antifungal activity of novel N-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine deriva- tives (5a-l) showed that most of the novel derivatives found to have greater activity on all the four fungal pathogens used. Compounds 5b, 5e, 5g and 5i found to be highly active against all the four fungal strains. Compound 5k showed moderate activity against Aspergillus niger, Aspergillus flavus, Candida albicans and low activity against the fungal starin, Neurospora crassa. The results are tabulated in table 2 and schematically depicted in figure 2.

Table 2: Antibacterial activity results (Zone of inhibition) of novel *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amines (5a-l) against Gram +ve and Gram –ve bacteria and ampicillin (Ref. Std) .

Compound Code	Diameter of inhibition zone in mm*
	Bacterial strains

	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*P.vulgaris*
5a	24.5	27.5	26.5	20.25	22.0	21.5
5b	20.0	21.0	18.25	17.0	18.0	17.5
5c	15.0	17.0	14.0	12.0	< 9.5	NA
5d	17.0	18.5	16.5	15.0	12.5	14.0
5e	16.25	17.0	<10.0	NA	13.6	15.8
5f	18.25	20.0	19.5	18.0	17.25	17.0
5g	22.55	23.0	24.0	20.5	18.5	20.0
5h	14.0	12.5	NA	15.0	14.5	NA
5i	20.0	23.5	21.0	20.5	20.0	19.5
5j	15.5	17.25	15.0	12.5	10.0	< 9.0
5k	18.25	19.0	20.0	18.25	19.5	16.25
5l	12.0	14.5	10.5	NA	< 8.0	NA
Standard (Ampicillin)	26.0	25.0	25.5	23.0	22.0	22.5

The reference standard, ampicillin was highly active against Gram +ve and Gram –ve bacterial species. However, tested compounds cannot directly be compared with the reference standard for their efficacy since it depends on therapeutic index, which varies for different bioactive compounds depending upon the ratio between lethal dose and therapeutic dose.

4. Conclusion

In our present investigation, we have designed a new series of N-substituted-(2,4-dichloro- phenyl)-1,3-thiazol-2-amine deriva- tives (5a-l) and evaluated for their in vitro antibacterial and antifungal activity against different pathogens. Among tested, compound 5a, 5f, 5g and 5i showed a potential bacterial inhibition property against both Gram positive and Gram negative species in comparison with the ref.std, ampicillin. Hence these compounds can be considered as potential antibacterial agents for the diseases caused by bacteria and the same compounds exhibited good fungal inhibitors against the ref.std, Nystatin which is used to study the antifungal activity.

5. Experimental Section

All the chemicals used in the synthesis were purchased from commercial suppliers and were used without further purification unless otherwise noted.. The purity of synthesized compounds was checked by TLC observing single spot on Merck silica gel 60 F254 coated alumina plates. The melting points of novel N-substituted-(2,4-dichloro- phenyl)-1,3-thiazol-2-amine deriva- tives (5a-l) were determined in open capillary tubes and are uncorrected. The IR spectra were obtained on Shimadzu-FTIR 577 Infrared spectrophotometer in KBr pellets. The 1H and 13C NMRspectra were recorded on Bruker FT-400 MHz spectrometer in CDCl3 and DMSO-d6 with

TMS as internal standard. The Mass spectra were recorded on Perkin-Elmer 018444–Y, Triple Quadruple LC/MS Spectrometer and elemental analyses were carried out using Heraus CHN rapid analyzer.

5. 1. Synthesis of 1-(2,4-dichlorophenyl)thiourea (3):

The starting material 1-(2,4-dichlorophenyl)thiourea **(3)** was prepared in the laboratory via condensation of equimolar quantities of 2,4-dichloroaniline **(1)** (0.1molar) and ammonium thiocyanate **(2)** (0.1molar) . The reaction mixture was refluxed for 8-10 hours in ethanol as a solvent. The formation of the product was confirmed by running TLC in ethylacetate-petroleum ether medium, observed single spot. The resulting mixture was poured into crushed ice, a solid separated, filtered, dried and the white crystalline solid was recrystallized using hot ethanol (**Scheme- I).**

5. 2 Synthesis of novel *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives (5a-l).

The target compounds, *N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives **(5a-l)** was synthesized by the refluxing 1-(2,4-dichlorophenyl)thiourea **(3)** (1.1055g, 0.005mol) with different phenacyl halides **(4)** (0.005mol) for 16 hours at 80^0C (**Scheme-II).** The reaction mixture was poured into crushed ice, filtered, dried and recrystallized by hot ethanol. The product was purified by silica gel column chromatography using hexane-ethyl acetate eluent The novel N-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amines**(5a-l)** obtained in good yield as white crystalline solids and structures of all the synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, LC-Mass spectra and Elemental analyses and evaluated for their antimicrobial properties. The characterization data are incorporated in the **Table 1.**

The spectral interpretations and elemental analyses of all the synthesized ***N*-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amine derivatives (5a-l)** are given below**:**

5.2.1.1. *N*-(2,4-dichlorophenyl)-4-(naphthalen-2-yl)-1,3-thiazol-2-amine (5a):

IR (KBr, cm^{-1}): Yield: 79%, m.p.: 111-113 ºC, IR (KBr, γ_{max}, cm^{-1}): 3383(>NH), 3026 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1548 (>C=N stretch), 1517, 1458, 1305 (>C=C<), 1214(>C-S), 808 and 738 (C-Cl).^{1}H-NMR(400MHz, DMSO, δ ppm): 8.40 (s, 1H, -NH), 7.95 (s, 1H, thiazole ring), 7.92 (d, 1H, J = 8.4Hz, 2,4-dichlorophenyl moiety), 7.88 (d, 1H, J = 8.4Hz, 2,4-dichlorophenyl moiety) , 7.60 – 7.49 (m, 7H, napthyl). ^{13}C-NMR (□ ppm): 121.72, 122.73, 123.98, 124.23, 126.39, 127.55, 128.12 and 128.17 (10 C-atoms of naphthyl moiety), 128.83, 131.77, 132.46, 133.15, 136.79 and 127.92 (6 C–atom of 2,4-dichlorophenyl moiety), 105.72, 149.62 and 163.26 (3 C-atoms of thiazole ring). LC-Mass, [M^+], (m/z): 370.84/372.84/373.64. Anal. calcd. (found) % for $C_{19}H_{12}Cl_2N_2S$: C, 61.23 (61.46); H, 3.98 (3.26); N, 7.89 (7.55).

5.2.1.2. 4-(4-bromophenyl)-*N*-(2,4-dichlorophenyl)-1,3-thiazol-2-amine (5b):

Yield: 74%, m.p.: 95-97 ºC. IR (KBr, γ_{max}, cm^{-1}): 3375(>NH), 3031 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1537 (>C=N stretch), 1301(>C-S), 820 and 813 (C-Cl), 665 (C-Br). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.81 (s, 1H, -NH), 8.51 (s, 1H, thiazole ring), 7.84(d, 2H, J = 8.8Hz, 4-bromophenyl moiety), 7.61 (d, 2H, J = 8.8Hz, 4-bromophenyl moiety) , 7.64 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.43 d, 1H, J = 8.8, 2,4-dichlorophenyl moiety). ^{13}C-NMR (□ ppm): 121.43, 122.69, 126.30 and 127.82 (6C-atoms of 4-bromophenyl moiety), 123.70, 125.21, 127.91, 128.89, 128.85 and 128.93 (6C–atom of 2,4-dichlorophenyl moiety), 106.00, 152.15 and 165.27(3C-atoms of thiazole ring). LC-Mass, [M^+], (m/z): 400.97/398.07/ 402.97/404.97. Anal. calcd. (found) % for $C_{15}H_9N_2SBrCl_2$: C, 45.65 (45.03); H, 2.54 (2.27); N, 7.73 (7.00).

5.2.1.3. *N*-(2,4-dichlorophenyl)-5-methyl-4-phenyl-1,3-thiazol-2-amine (5c):

Yield: 74%, m.p.: 95-97 ºC. IR (KBr, γ_{max}, cm^{-1}): 3385(>NH), 3014 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 2952 (methyl), 1545 (>C=N stretch), 1258(>C-S), 831 and 815 (C-Cl).^{1}H-NMR(400MHz, DMSO, δ ppm): 9.83 (s, 1H, -NH), 8.59 (s, 1H, thiazole ring), 7.89(d, 2H, J = 8.4Hz, phenyl moiety), 7.58 (d, 2H, J = 8.4Hz, phenyl moiety) , 7.41 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.38 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety). 2.44 (s, 3H, methyl group). ^{13}C-NMR (□ ppm): 20.56 (- CH_3), 124.25, 126.98, 128.38 and 130.74 (6 C-atoms of phenyl moiety), 131.85, 1, 131.96, 133.25, 134.61, 135.63 and 136.43(6 C–atom of 2,4-dichlorophenyl moiety), 108.39, 149.82 and 167.08(3 C-atoms of thiazole ring). LC-Mass, [M^+], (m/z): 349.17/345.61/343.25/341.02. Anal. Calcd for $C_{17}H_{14}Cl_2N_2S$: C, 58.69; H, 4.56; N, 8.74. Found: C, C, 58.69 (58.46); H, 4.56 (4.04); N, 8.74 (8.04).

5.2.1.4. 4-(4-bromophenyl)-*N*-(2,4-dichlorophenyl)-5-methyl-1,3-thiazol-2-amine (5d):

Yield: 68%, m.p.: 88-90 ºC. IR (KBr, γ_{max}, cm^{-1}): 3394(>NH), 3063 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 2929 (methyl), 1569 (>C=N stretch), 1269 (>C-S), 842 and 827 (C-Cl), 602(C-Br). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.79 (s, 1H, -NH), 8.56 (s, 1H, thiazole ring), 7.84(d, 2H, J = 8.8Hz, 4-bromophenyl moiety), 7.61 (d,

2H, J = 8.8Hz, 4-bromophenyl moiety) , 7.46 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.43 d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 2.93 (s, 3H, methyl group). ^{13}C-NMR (δ ppm): 21.25 (- $\underline{C}H_3$), 121.30, 122.54, 123.86 and 124.45 (6 $\underline{C}$-atoms of 4-bromophenyl moiety), 126.21, 126.85, 127.16, 127.57, 128.71 and 129.00(6 $\underline{C}$–atom of 2,4-dichlorophenyl moiety), 106.70, 153.24 and 164.31(3 $\underline{C}$-atoms of thiazole ring). LC-Mass, [M$^+$], (m/z): 414.28/412.87/410.11/408.69. Anal. calcd. (found) % for $C_{16}H_{11}N_2SBrCl_2$: C, 46.52 (46.40); H, 2.71 (2.68); N, 6.31 (6.76).

5.2.1.5. *N*-(2,4-dichlorophenyl)-4-(3,4-dich

Yield: 81%, m.p.: 78-80 ºC. IR (KBr γmax, cm^{-1}): 3371(>NH), 3027 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1590 (>C=N stretch), 1258(>C-S), 836, 801, 752 and 716 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.70 (s, 1H, -NH), 8.14 (s, 1H, thiazole ring), 7.78(d, 1H, J = 8.8Hz, 3,4-dichlorophenyl moiety), 7.68 (d, 1H, J = 8.8Hz, 3,4-dichlorophenyl moiety), 7.54 (d, 1H, J = 8.8, 3,4-dichlorophenyl moiety), 7.27 (d, 1H, J = 8.4, 2,4-dichlorophenyl moiety), 7.19 (d, 1H, J = 8.4, 2,4-dichlorophenyl moiety).). ^{13}C-NMR (δ ppm): 126.35, 126.98, 127.58, 127.36, 131.85 and 133.67 (6 $\underline{C}$-atoms of 3,4-dichlorophenyl moiety), 134.57, 134.29, 134.74, 135.91, 136.48 and 137.19(6 $\underline{C}$–atom of 2,4-dichlorophenyl moiety), 105.49, 148.79 and 161.55(3 $\underline{C}$-atoms of thiazole ring). LC-Mass, [M$^+$], (m/z): 390.24/388.89/386.95/383.99. Anal. calcd. (found) % for $C_{16}H_{11}N_2SBrCl_2$: C, 46.89 (46.18); H, 2.83 (2.07);N, 7.41 (7.18).

5.2.1.6. 4-(biphenyl-4-yl)-*N*-(2,4-dichlorophenyl)-1,3-thiazol-2-amine (5f):

Yield: 72%, m.p.: 158-160 ºC. IR (KBr, γmax, cm^{-1}): 3379 (>NH), 3050 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1510 (>C=N stretch), 1305(>C-S), 798 and 760 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 8.95 (s, 1H, -NH), 8.31 (s, 1H, thiazole ring), 7.64(d, 2H, J = 8.4Hz, phenyl moiety), 7.52 (d, 2H, J = 8.4Hz, phenyl moiety), 7.48 (d, 2H, J = 8.4Hz, 4-phenyl moiety), 7.36 (d, 1H, J = 8.4Hz, 4-phenyl moiety), 7.38 (t, 1H, J = 8.0Hz, 4-phenyl moiety), 7.10 (d, 1H, 2,4-dichlorophenyl moiety), 7.04 (d, 1H, 2,4-dichlorophenyl moiety).). ^{13}C-NMR (δ ppm): 127.63, 127.96, 128.20, 128.48, 129.59, 131.43 and 132.80 (12 $\underline{C}$-atoms of 4-phenyl moiety), 120.10, 125.72, 126.56, 127.87, 131.27 and 134.67(6 $\underline{C}$–atom of 2,4-dichlorophenyl moiety), 103.33, 149.75 and 160.51(3 $\underline{C}$-atoms of thiazole ring). LC-Mass, [M$^+$], (m/z): 397.05/395.25/393.52. Anal. calcd. (found) % for $C_{21}H_{14}N_2SCl_2$: C, 63.59 (63.48); H, 3.45 (3.55); N, 7.63 (7.05).

5.2.1.7. *N*, 4-bis(2,4-dichlorophenyl)-1,3-thiazol-2-amine (5g):

Yield: 76%, m.p.: 132-134 ºC, IR (KBr, γmax, cm^{-1}): 3400(>NH), 3060 and 3049 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1543 (>C=N stretch), 1303(>C-S), 813, 731 and 692 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.82 (s, 1H, -NH), 7.39 (s, 1H, thiazole ring), 8.59(d, 1H, J = 9.2Hz, 2,4-dichlorophenyl moiety), 7.97 (d, 2H, J = 8.4Hz, 2,4-dichlorophenyl moiety) , 7.72 (d, 1H, J = 8.0Hz, 2,4-dichlorophenyl moiety), 7.71(d, 1H, J = 7.2Hz, 2,4-dichlorophenyl moiety), 7.36 (d, 1H, J = 7.2Hz, 2,4-dichlorophenyl moiety). ^{13}C-NMR (δ ppm): 121.72, 122.47, 123.86, 124.32, 125.46 and 126.18(6 $\underline{C}$-atoms of 2,4-chlorophenyl moiety), 126.26, 127.52, 127.63, 128.76, 129.08 and 129.21 (6 $\underline{C}$–atom of 2,4-dichlorophenyl moiety), 107.00, 152.05 and 163.19(3 $\underline{C}$-atoms of thiazole ring). LC-Mass, [M$^+$], (m/z): 390.10/392.10/394.10. Anal. calcd. (found) % for $C_{15}H_8N_2SCl_4$, C, 46.01 (46.48); H, 2.52 (2.07); N, 7.23 (7.18)

5.2.1.8. *N*-(2,4-dichlorophenyl)-4-[4-(methylsulfonyl)phenyl]-1,3-thiazol-2-amine (5h):

Yield: 73%, m.p.: 116-120 ºC. IR (KBr, γmax, cm^{-1}): 3295(>NH), 3005 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 2968 (methyl), 1570 (>C=N stretch), 1261(>C-S), 1312 and 1167 (asymmetric and symmetric stretch, O=S=O group), 801, and 736 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.36 (s, 1H, -NH), 8.37 (s, 1H, thiazole ring), 7.74(d, 2H, J = 8.4Hz, -SO_2CH_3phenyl group), 7.41 (d, 2H, J = 8.4Hz, -SO_2CH_3phenyl group), 7.27(d, 1H, J = 8.0Hz, 2,4-dichlorophenyl moiety), 7.05 (d, 1H, J = 8.0Hz, 2,4-dichlorophenyl moiety). ^{13}C-NMR (δ ppm): 44.99 (-$SO_2\underline{C}H_3$), 128.5, 128.87, 138.31 and 138.82 (6 $\underline{C}$-atoms of 4- $SO_2\underline{C}H_3$ phenyl moiety), 122.85, 125.63, 127.56, 157.68, 133.4 and 134.28(6 $\underline{C}$–atom of 2,4-dichlorophenyl moiety), 104.27, 149.73 and 161.31(3 $\underline{C}$-atoms of thiazole ring). LC-Mass, [M$^+$], (m/z): 400.15/ 398.54/396.17/394.08. Anal. calcd. (found) % for $C_{16}H_{12}O_2N_2S_2Cl_2$: C, 48.51 , (48.13); H, 3.40 (3.03); N, 7.09 (7.02)

5.2.1.9. *N*-(2,4-dichlorophenyl)-4-(4-fluorophenyl)-1,3-thiazol-2-amine(5i):

Yield: 80%, m.p.: 112-114 ºC. IR (KBr, γmax, cm^{-1}): 3262(>NH), 3018 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1517 (>C=N stretch), 1249(>C-S), 1052 (C-F), 815 and 748 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.79 (s, 1H, -NH), 8.05 (s, 1H, thiazole ring), 7.67(d, 2H, J = 8.8Hz, 4-flourophenyl moiety), 7.58 (d, 2H, J = 8.8Hz, 4-flourophenyl moiety) , 7.40 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.38 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety). ^{13}C-NMR (δ ppm): 128.36, 128.96, 129.25 and 130.30 (6 $\underline{C}$-atoms of 4-flourophenyl moiety), 122.38,

126.28, 127.02, 127.94, 131.76 and 132.61(6 C–atom of 2,4-dichlorophenyl moiety), 107.23, 148.84 and 162.75(3 C-atoms of thiazole ring). LC-Mass, [M+], (m/z): 339.34/337.46/335.19. Anal. calcd. (found) % for $C_{15}H_9N_2SCl_2F$: C, 53.60 (53.11); H, 2.60 (2.67); N, 8.21 (8.26).

5.2.1.10. *N*-(2,4-dichlorophenyl)-4,5-dimethyl-1,3-thiazol-2-amine (5j):

Yield: 71%, m.p.: 102-104 ºC, IR (KBr, γ_{max}, cm^{-1}): 3227(>NH), 3040 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 2918, 2906 (methyl groups), 1510 (>C=N stretch), 1279(>C-S), 1052 (C-Cl), 838 and 796 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.02 (s, 1H, -NH), 8.12 (s, 1H, thiazole ring), 7.50 (d, 1H, J = 8.4, 2,4-dichlorophenyl moiety), 7.21(d, 1H, J = 8.4, 2,4-dichlorophenyl moiety), 2.37 (s, 3H, methyl group), 2.31(s, 3H, methyl group). ^{13}C-NMR (□ ppm): 18.21 and 19.36 (2 C-atoms of two CH_3 groups), 119.56, 124.38, 126.98, 127.46, 131.07 and 133.48 (6 C–atom of 2,4-dichlorophenyl moiety), 115.56, 146.8 and 158.73(3 C-atoms of thiazole ring). LC-Mass, [M+], (m/z): 272.87/274.54/272.06. Anal. calcd. (found) % for $C_{11}H_{10}N_2SCl_2$: C, 48.41 (48.36); H, 3.23 (3.69); N, 10.25 (10.28).

5.2.1.11. *N*-(2,4-dichlorophenyl)-4-(trifluoromethyl)-1,3-thiazol-2-amine (5k):

Yield: 73%, m.p.: 108-110 ºC. IR (KBr, γ_{max}, cm^{-1}): 3262(>NH), 3012 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1532 (>C=N stretch), 1250 (>C-S), 998 (C-F), 784 and 773 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 8.87 (s, 1H, -NH), 8.01 (s, 1H, thiazole ring), 7.28 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.16 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 3.84 (s, -CF_3 group). ^{13}C-NMR (□ ppm): 120.45 (-CF_3), 128.41, 128.64, 129.89, 129.42, 130.76 and 133.27 (6 C–atom of 2,4-dichlorophenyl moiety), 106.91, 139.62 and 161.24(3 C-atoms of thiazole ring). LC-Mass, [M+], (m/z): 313.65/311.46/309.87. Anal. calcd. (found) % for $C_{10}H_5N_2SCl_2F_3$: C, 38.43 (38.36) H, 1.59 (1.61); N, 8.90 (8.95).

5.2.1.12. *N*-(2,4-dichlorophenyl)-5-methyl-4-phenyl-1,3-thiazol-2-amine (5*l*):

Yield: 75%, m.p.: 68-70 ºC. IR (KBr, γ_{max}, cm^{-1}): 3295(>NH), 3057 (Ar. C-H stretch, 2,4-dichlorophenyl moiety), 1550 (>C=N stretch), 1278 (>C-S), 880, 782 and 763 (C-Cl). ^{1}H-NMR(400MHz, DMSO, δ ppm): 9.18 (s, 1H, -NH), 7.77(d, 2H, J = 8.4Hz, 4-phenyl moiety), 7.60 (d, 2H, J = 8.8Hz, 4-phenyl moiety) , 7.53 (t, 1H, J = 8.0Hz, 4-phenyl moiety), 7.39 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety), 7.24 (d, 1H, J = 8.8, 2,4-dichlorophenyl moiety). ^{13}C-NMR (□ ppm): 128.54, 128.27, 129.07 and 130.34(6 C-atoms of phenyl moiety), 131.25, 131.47, 132.81, 132.45, 133.43 and 133.99(6 C–atom of 2,4-dichlorophenyl moiety), 110.22, 156.50 and 160.11(3 C-atoms of thiazole ring). LC-Mass, [M+], (m/z): 356.11/354.49/352.32/350.27. Anal. calcd. (found) % for $C_{15}H_9N_2SCl_3$: C, 50.44 (50.65); H, 2.50 (2.55); N, 7.18 (7.88).

Conflict of interest

The authors would like to declare there is no conflict of interest.

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Table 3: Antifungal activity results of novel *N*-substituted-(2,4-dichlorophenyl)-1,3- thiazol-2-amines(5a-l) against fungal strains and Nystatin (Ref.std).

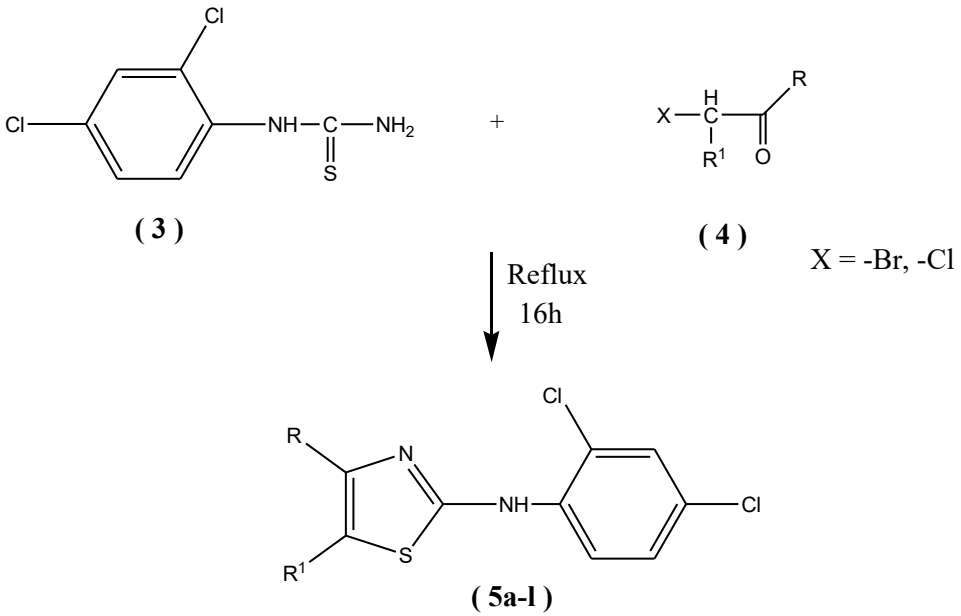
Compound Code	Diameter of inhibition zone in mm*			
	Fungal strains			
	Aspergillus niger	*Aspergillus flavus*	*Candida albicans*	*Neurospora crassa*
5a	18.0	16.5	22.5	15.0
5b	19.5	18.0	20.25	18.0
5c	14.0	14.0	15.5	11.5

5d	17.15	18.5	13.25	15.0
5e	10.5	10.0	NA	NA
5f	18.0	16.5	20.0	15.5
5g	18.5	19.0	22.5	18.0
5h	12.0	11.0	NA	NA
5i	19.0	17.5	20.5	18.25
5j	13.5	11.0	15.25	NA
5k	15.0	16.25	19.0	15.0
5l	< 8.0	NA	11.0	NA
Standard (Nystatin)	25.0	22.5	24.55	23.5

*Mean of the triplicate values NA: No activity

Antibacterial Activity (Zone of Inhibition) of N-substituted-(2,4-dichlorophenyl)-1,3-thiazol-2-amines(5a-l) and Ampicillin (Ref. standard) against Gram +ve and Gram –ve bacteria.

Reaction Schemes



R^1 = -H, $-CH_3$, -Cl,

R = 2,4-F_2-Ph, napthyl, 4-Br-Ph, Ph, 3,4--Cl_2-Ph, 4,4^1-biPh, 2,4-Cl_2-Ph, SO_2Me-Ph, 4-F-Ph, $-CH_3$, $-CF_3$,

Scheme 2

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