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Synthesis of 10-(2-Phenyl-imidazo [2, 1-b] [1,3,4]thiadiazol-6-yl)-10H-phenothiazine derivatives and their *In-vitro* Biological Studies

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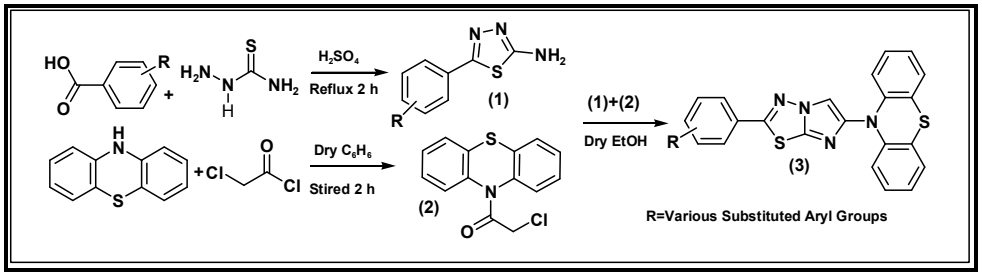
ABSTRACT

*New series of 10-(2-Styryl-5,6-dihydro-imidazo[2,1-b][1,3,4]thiadiazole-6-yl)-10H-phenothiazine were synthesized by cyclization of various carboxylic acid with thiosemicarbazide in presence of sulphuric acid was to get compound **1**. Another way phenothiazine treated with chloroacetyl chloride yielded compound **2**. Further, cyclization of compounds **1** and **2** followed by refluxation about 18 h to get the final products **3** and **3a–3i** of the series. The structures of compounds were confirmed by IR, ^{1}H-NMR, ^{13}C NMR and mass spectroscopy and by chemical analysis. All the above compounds were screened for their antimicrobial activity against some selected bacteria and fungi such as E. coli, B. subtilis, and S. typhi bacteria and A.niger, A. flavus and F. oxisporium fungi.*

Graphical Abstract



Keywords: Thiadiazole, Phenothiazine, Imidazole, Antimicrobial activities.

INTRODUCTION

In the past years, the literature is enriched with progressive finding about the synthesis and pharmacological actions of fused heterocycles [1]. In the field of synthetic organic chemistry nitrogen-sulphur heteroatom containing aromatic molecules particularly 10-*H*-phenothiazine and 1,3,4-thiadiazole are becoming more popular as an area of research and provides a most valuable molecular template for the development of new molecule that are able to interact with a wide variety

of biochemical processes. They have been shown to possess a broad spectrum of pharmacological activities such as anti-tubercular [2-3] anti-tumour [4] anti-inflammatory [5] antihyperlipidemic [6], cytotoxic [7], antimicrobial [8] and antiproliferative [9] agents. In continuation in the our aim synthesis of new bioactive molecule by incorporating phenothiazine and 1,3,4 heterothiadiazole moieties in a single molecular framework, both molecule have broad biological spectrum such as antibacterial [10-11], antifungal [12-13] anticancer [14-15], anticonvulsant [16-17] , antitubercular [18-19] and anti-inflammatory [20-21] herein, we carry out the synthesis and antimicrobial evaluation of some new synthesized molecule. Number of molecules have been claimed by researchers Imidazo [2,1-b]-1,3,4-thiadiazole all around the world because of its excellent biological profile. We have decided to synthesize a new series of 10-(2-Phenyl-imidazo [2,1-b][1,3,4]thiadiazol-6-yl)-10H-phenothiazine shown in Scheme 1. The starting material, thiosemicarbazide undergoes cyclodehydration of acyl thiosemicarbazides treated with in situ by heating the various carboxylic acid in presence of H_2SO_4 yielded compound **1**, 5-Phenyl-[1,3,4]thiadiazol-2-ylamine. In another separate reaction 10-H phenothiazine treated with chloroacetyl chloride yielded compound **2**, 2-Chloro-1-phenothiazin-10-yl-ethanone. Further condensation reaction of compound **2** and compound **1** under reflux in dry ethanol 18 h yielded compound **3**, 10-(2-Phenyl-imidazo[2,1-b][1,3,4]thiadiazol-6-yl)-10H-phenothiazine, further compounds (**3a–3i**) synthesized by similar method as reported earlier. The structure of compounds **1** and (**1a-1i)** , compound **2**, and compound **3** and **(3a–3i)** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass and chemical analysis. All the compounds **3 and (3a–3i)** were screened for their *in vitro* antimicrobial activity against some selected bacteria and fungi.

MATERIALS AND METHODS

All the chemicals and reagents were of analytical grade of sigma Aldrich, Merck, Chemi-loba and Himedia. The reagents and solvents were purified before using by standard methods. Melting points were taken in open capillaries and are uncorrected. Progress of reaction was monitored at various stages by silica gel-G coated TLC plates using MeOH: $CHCl_3$ system. The spot was visualized by exposing dry plate at iodine vapour chamber and fluorescent indicator F 254 UV chamber. IR spectra were recorded in KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (ν max in cm^{-1}) and ^{1}H NMR and ^{13}C NMR spectra were measured on a Brucker DRX-300 spectrometer in $CDCl_3$ at 500 and 75 MHz respectively using TMS as an internal standard. All chemical shifts were reported on δ scale. The mass spectra were recorded on a Jeol SX–102 GC-MS mass spectrometer. Elemental analyses were performed on a Carlo Erba–1108 analyzer. The analytical data of all the compounds were highly satisfactory. All the synthesized compounds were purified by column chromatography using Merck silica Gel 60 (230-400 Mesh). The reagent grade chemicals were purchased from the commercial sources.

Synthesis of 5-Phenyl-[1,3,4]thiadiazol-2-ylamine (1): Equimolar mixture of thiosemicarbazide (0.004 mole) and benzoic acid (0.004mole) in presence of H_2SO_4 in dry ethanol (25 ml) was refluxed on a water bath for about 2 h TLC was used to check reaction progress, then mixture was removed and poured in crushed ice to get a white precipitate, compound **1**. A solid product was obtained which was purified over a silica gel column using chloroform: methanol (8:2 v/v) mixture as eluant. The elute was concentrated to get a solid product which was recrystallized from ethanol to yielded compound **1**: White crystalline solid. M.P. 223-225 ^{0}C,Yield 70 %, IR(ν max cm^{-1}): 1430 (ν_{C-C}), 1070 (ν_{C-N}) 763 (ν_{C-S}), 1454 ($\nu_{C=C}$), 1585 ($\nu_{N=C}$), 3378 (ν_{-NH2}), ^{1}H NMR: δ(ppm) 4.87 (2H, s, NH_2) , 7.29 -7.73 (5H, m, Ar-H), ^{13}C NMR : δ (ppm)126.9-131.01(C of aromatic ring), 169.4,163.8(C_2,C_5 of thiadiazole ring),Anal. Calcd. for $C_8H_7N_3S$:C, 54.22, H, 3.98, N, 23.71 % found C, 54.09, H, 3.70, N, 23.40 % ; MS 177.03 (M^+).

The compounds **1a-1i** was synthesized by the similar method as reported earlier.

5-(2-chloro-phenyl)-[1,3,4]thiadiazole-2-ylamine (1a): M.P.229-230°C, Yield 72%, IR (ν max cm^{-1}): 1433(ν_{C-C}), 1072 (ν_{C-N}), 780 (ν_{C-S}),1541($\nu_{C=C}$), 1587 ($\nu_{N=C}$), 745 (ν_{C-Cl}) 3380 (ν-NH_2), ^{1}H NMR δ(ppm) 4.73(2H, s, NH_2)7.27-8.18 (4H, m, Ar-H), ^{13}C NMR: δ(ppm) 127.5-133.1(C of aromatic

ring),163.3,169.4(C_2C_5 of thiadiazole ring), Anal. Calcd. for C_8H_6 ClN_3S: C, 45.39, H, 2.86, N,19.85% found C,45.09,H, 2.70, N,19.40%; MS 211.01 (M^+).

5-(3-chloro-phenyl)-[1,3,4]thiadiazole-2-ylamine(1b): M.P.228-230°C, Yield 69%, IR(ν max cm^{-1}): 1429 (ν_{C-C}), 1069 (ν_{C-N}), 779 (ν_{C-S}), 1537($\nu_{C=C}$), 1587 ($\nu_{N=C}$), 737 (ν_{C-Cl}) 3382 (ν_{-NH2}), ^{1}HNMR: δ(ppm) 4.75 (2H, s, NH_2), 7.35-7.68 (4H, m, Ar-H),^{13}C NMR : δ (ppm) 126.91-31.01 (C of aromatic ring),169.2,162.9(C_2C_5 of thiadiazole ring), Anal. Calcd. for C_8H_6 ClN_3S: C,45.39,H, 2.86,N, 19.85 % found C, 45.19,H, 2.65, N, 19.49%; MS 211.0 (M^+)

5-(4-chloro-phenyl)-[1,3,4]thiadiazole-2-ylamine(1c): M.P.230-232°C,Yield73%, IR (ν max cm^{-1}): 1427 (ν_{C-C}), 1050 (ν_{C-N}), 781 (νC-S), 1539 ($\nu_{C=C}$), 1588 ($\nu_{N=C}$), 749 (ν_{C-Cl}),3383 (ν_{-NH2}), ^{1}HMNR: δ(ppm) 4.89 (2H, s, NH_2), 7.73-7.85 (4H, m, Ar-H), ^{13}C NMR: δ (ppm) 129.1-135.6 (C of aromatic ring), 163.4,168.8(C_2C_5 of thiadiazole ring), Anal. Calcd. for C_8H_6 ClN_3S: C,45.39,H, 2.86,N, 19.85% found C, 45.19,H,2.61, N, 19.38% ; MS 211.02 (M^+).

5-(2-bromo-phenyl)-[1,3,4]thiadiazole-2-ylamine (1d): M.P.228-230°C,Yield 68%, IR (ν max cm^{-1}): 1426 (ν_{C-C}),1055 (ν_{C-N}), 768 (ν_{C-S}), 1545 ($\nu_{C=C}$), 1640 ($\nu_{N=C}$), 545 (ν_{C-Br}) 3386 (ν_{-NH2}), ^{1}HNMR: δ(ppm) 4.80 (2H, s, NH_2) 7.25-7.89 (4H, m, Ar-H), ^{13}C NMR : δ (ppm) 163.1,169.5(C_2 C_5 of thiadiazole ring), 120.7- 132.1(C of aromatic ring), Anal. Calcd. for $C_8H_6BrN_3S$: C, 37.52, H, 2.36, N, 16.41% found C, 37.18,H, 2.21, N, 16.35%; MS 254.94 (M^+).

5-(3-bromo-phenyl)-[1,3,4]thiadiazole-2-ylamine (1e): M.P. 229-230°C, Yield 67%, IR (ν max cm^{-1}): 1431 (ν_{C-C}), 1052 (ν_{C-N}), 767 (ν_{C-S}), 1540 ($\nu_{C=C}$), 1646 ($\nu_{N=C}$), 536 (ν_{C-Br}), 3388 (ν_{-NH2}), ^{1}H NMR: δ(ppm) 4.84 (2H, s, NH_2), 7.31-7.64 (4H, m, Hz, Ar-H), ^{13}C NMR: δ (ppm): 118.1-132.9(C of aromatic ring),164.08,168.9(C_2 C_5 of thiadiazole ring), Anal. Calcd. for $C_8H_6BrN_3S$: C, 37.52,H, 2.36,N, 16.41% found C, 37.28,H, 2.24, N, 16.25 % ; MS 254.82 (M^+).

5-(4-bromo-phenyl)-[1,3,4]thiadiazole-2-ylamine (1f): M.P. 231-233°C, Yield 69%, IR (ν max cm^{-1}): 1429 (νC-C), 1041 (ν_{C-N}), 766 (ν_{C-S}), 1543 ($\nu_{C=C}$), 1642 ($\nu_{N=C}$), 541(ν_{C-Br}), 3390 (ν_{-NH2}), ^{1}H NMR: δ(ppm) 4.79(2H, s, NH_2) ,7.68-7.79(4H, m, Ar-H), ^{13}C NMR :δ (ppm) 124.0-131.0 (C of aromatic ring), 164.1-169.5(C_2 C_5 of thiadiazole ring), Anal. Calcd. for $C_8H_6BrN_3S$: C, 37.52 ,H, 2.36,N, 16.41% found C, 37.24, H, 2.20, N, 16.35% ; MS 254 (M^+).

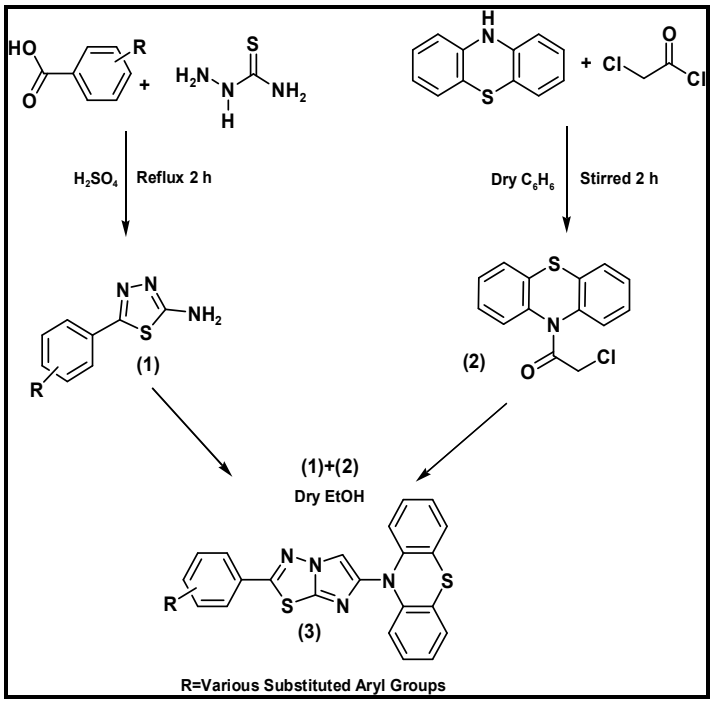
5-(2-nitro-phenyl)-[1,3,4]thiadiazole-2-ylamine (1g): M.P. 257-259°C, Yield 78%, IR (ν max cm^{-1}): 1428 (ν_{C-C}), 1053 (ν_{C-N}), 778 (ν_{C-S}), 1515 ($\nu_{C=C}$), 1651 ($\nu_{N=C}$), 1341(ν_{C-NO2}), 3391 (-ν_{NH2}), ^{1}H NMR: δ(ppm) 4.90 (2H, s, NH_2), 7.59-8.27 (4H, m, Ar-H), ^{13}C NMR : δ (ppm) 127.5-148.3(C of aromatic ring), 164.01, 169.6(C_2C_5 of thiadiazole ring), Anal. Calcd. for $C_8H_6N_4O_2S$: C, 43.24, H, 2.72, N, 25.21% found C,43.14,H, 2.52, N, 25.8%; MS 222.02 (M^+).

5-(3-nitro-phenyl)-[1,3,4]thiadiazole-2-ylamine (1h): M.P. 259-261°C, Yield 80%, IR: (ν max cm^{-1}): 1426 (ν_{C-C}), 1048 (ν_{C-N}), 776 (ν_{C-S}), 1527 ($\nu_{C=C}$), 1656 ($\nu_{N=C}$), 1343 (ν_{C-NO2}), 3393 (-ν_{NH2}),^{1}H NMR: δ(ppm) 4.78 (2H, s, NH_2), 7.59-7.91 (4H, m, Ar-H),^{13}CNMR: δ (ppm) 116.3-140.4 (C of aromatic ring), 164.2 169.3 (C_2 C_5 of thiadiazole ring), Anal. Calcd. for $C_8H_6N_4O_2S$: C, 43.24, H, 2.72, N, 25.21% found C, 43.16,H, 2.62, N, 25.10%; MS 222.22 (M^+).

5-(4-nitro-phenyl)-[1,3,4]thiadiazole-2-ylamine(1i): M.P. 258-260°C, Yield 79%, IR(ν max cm^{-1}): 1432 (ν_{C-C}), 1055(ν_{C-N}), 771 (ν_{C-S}), 1522 ($\nu_{C=C}$), 1655 ($\nu_{N=C}$), 1340 (ν_{C-NO2}), 3395 (ν_{-NH2}), ^{1}H NMR: δ(ppm) 4.81(2H, s, NH_2) , 7.71-8.27 (4H, m, Ar-H), ^{13}C NMR: δ (ppm)117.2-140.4(C of aromatic ring), 164.2-168.8 (C_2C_5 of thiadiazole ring), Anal. Calcd. for $C_8H_6N_4O_2S$: C, 43.24, H, 2.72, N, 25.21% found C, 43.26,H, 2.60, N, 25.12%; MS 222.19 (M^+).

Synthesis of 2-Chloro-1-phenothiazin-10-yl-ethanone (2): Chloroacetyl chloride (0.06 mol) was added drop wise at 0.5°C to phenothiazine (0.06 mol) in dry benzene (100 mL) and the mixture was stirred for 2 h. Reaction progress was checked by TLC during the reaction. After the completion of

the reaction, the benzene was distilled off to get a solid product washed with petroleum ether which was purified over a silica gel column using chloroform: methanol (8:2 v/v) mixture as eluant. The elute was concentrated to give a product which was recrystallized from ethanol to yielded compound **2**. M.P.190-192°C, Yield 94%, IR: (ν max cm^{-1}) 1470 (ν_{C-C}), 2936 (ν_{C-H}), 1333(ν_{N-C}), 1552 ($\nu_{C=C}$), 2836 (ν_{-CH2}),1671($\nu_{C=O}$), 685 (ν_{C-S-C}),735(ν_{C-Cl}). ^{1}H NMR: δ(ppm) 4.35(2H, s acyclic CH_2), 7.14-7.40 (8H, m, Ar-H),^{13}C NMR δ (ppm) 123.1-138.8 (C of phenothiazine ring), 165.5(C=O acyclic), 42.2 (CH_2 acyclic), Anal. Calcd. for $C_{14}H_{10}ClNOS$: C, 60.98; H, 3.66, N, 5.08, found C, 60.76,H, 3.50, N, 5.01, MS 275.02 (M^+).



Reaction Scheme

10-(2-Phenyl-imidazo[2,1-b][1,3,4]thiadiazol-6-yl)-10H-phenothiazine (3): Equimolar amount of 5-Phenyl-[1,3,4]thiadiazol-2-ylamine, compound **1** (0.004 Mole) and Chloro-1-phenothiazin-10-yl-ethanone, compound **2** (0.004 mol) in ethanol (20 mL) was refluxed on a water bath for about 18 h. After the completion of the reaction, the ethanol was distilled off to get a solid product which was purified over a silica gel column using chloroform: methanol (8:2 v/v) mixture as eluant. The elute was concentrated to give a product which was recrystallized from ethanol to yielded compound **3**.
Light green crystalline solid, M.P. 210-212^{0}C, Yield 70%, IR: (ν max cm^{-1}) 1481 (νC-C), 3171(ν_{C-H}),1638($\nu_{C=N}$ thiadiazole),1589($\nu_{C=N}$ imidazole),1286(ν_{N-C}),772(ν_{C-S}),1495 ($\nu_{C=C}$), 681 (ν_{C-S-C} phenothiazine). ^{1}H NMR:δ(ppm) 6.77(1H, s, imidazole),7.1-7.4 (8H, m, Ar-H phenothiazine),7.45-7.46 (3H, m Ar-H thiadiazole),8.00(2H, d, J = 8.0, Hz, Ar-H), ^{13}C NMR: δ(ppm) 125.9-130.4(C of aromatic ring), 124.2, 144.0 and 116.6-128.1(C of phenothiazine), 175.2,164.4 (C_2,C_5 thiadiazole), 100.9 and 150.6(C of imidazole), Anal. Calcd. for $C_{22}H_{14}N_4S_2$: C, 61.03, H, 3.03, N, 12.94 %, found C, 61.00, H,3.01, N, 12.74% ; MS 398.06 (M^+).

The compounds **3a-3i** was synthesized by the similar method as reported earlier.
10-[2-(2-Chloro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine(3a): M.P. 209-211°C,Yield 71%, IR: (νmax cm^{-1})1479 (ν_{C-C}), 3172(ν_{C-H}), 1640 ($\nu_{C=N}$ thiadiazole), 1597($\nu_{C=N}$ imidazole),1280(ν_{N-C}), 772(ν_{C-S}), 741(ν_{C-Cl}), 1493($\nu_{C=C}$),682(ν_{C-S-C} phenothiazine). ^{1}H NMR: δ(ppm)6.71(1H,s, imidazole), 7.11-7.45 (8H, m, Ar-H phenothiazine), 7.40-7.76 (4H,m, Ar-H

aromatic ring),^{13}C NMR:δ (ppm) 127.6-133.2 (C of aromatic ring), 124.4,144.2 and 116.7-129.3 (C of phenothiazine),164.7,156.3 (C_2,C_5 thiadiazole), 100.8 and 150.4 (C of imidazole), Anal. Calcd. for $C_{22}H_{13}Cl\ N_4\ S_2$, C, 61.03, H, 3.03, N,12.94 %, found C, 61.00, H, 3.01, N, 12.74%; MS 432.03(M^+).

10-[2-(3-Chloro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3b): M.P. 210-211°C, Yield 72%, IR:(ν max cm^{-1})1482 (ν_{C-C}), 3169 (ν_{C-H}), 1639 ($\nu_{C=N}$ thiadiazole), 1598 ($\nu_{C=N}$ imidazole), 1284(ν_{N-C}),77(ν_{C-S}),737(ν_{C-Cl}),1491($\nu_{C=C}$),679 (ν_{C-S-C} phenothiazine). ^{1}H NMR: δ(ppm) 6.76(1H, s, imidazole), 7.42-7.71 (3H, m,Ar-H aromatic ring), 7.11-7.44 (8H, m Ar-H penothiazine), 7.95 (1H, t, J = 1.5, 0.4 Hz, Ar-H), ^{13}C NMR: δ (ppm)126.9-131(C of aromatic ring), 124.1,145.1 and 116.6-128.0(C of phenothiazine), 157.7,164.3 (C_2,C_5 thiadiazole), 100.7 and 150.7 (C of imidazole), Anal. Calcd. for $C_{22}H_{13}\ ClN_4S_2$, C, 61.03, H, 3.03, N, 12.94%, found C, 61.01, H, 3.00, N, 12.84%; MS 432.02 (M^+).

10-[2-(4-Chloro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3c): M.P. 212-214°C, Yield 70%, IR:(νmax cm^{-1}) 1480 (ν_{C-C}), 3176 (ν_{C-H}), 1638 ($\nu_{C=N}$ thiadiazole), 1589 ($\nu_{C=N}$ imidazole), 1285 (ν_{N-C}), 770 (ν_{C-S}), 742 (ν_{C-Cl}), 1488 ($\nu_{C=C}$), 682 (ν_{C-S-C} phenothiazine).^{1}H NMR:δ(ppm) 6.79(1H, s, imidazole),7.68-7.70 (4H, m,Ar-H aromatic ring), 7.12-7.46 (8H, m Ar-H phenothiazine), ^{13}C NMR : δ (ppm) 127.2-135.7 (C of aromatic ring), 124.4,145.3 and 116.1-128.3(C of phenothiazine),157.7,156.3 (C_2,C_5 thiadiazole), 100.6 and 150.3 (C of imidazole), Anal. Calcd. for $C_{22}\ H_{13}\ Cl\ N_4S_2$,C, 61.03, H ,3.03, N, 12.94%, found C, 61.00, H, 3.02, N, 12.72%; MS 432.05 (M^+).

10-[2-(2-Bromo-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3d): M.P. 211-212°C, Yield 70%, IR: (νmax cm^{-1}) 1478 (ν_{C-C}),3175 (ν_{C-H}),1637($\nu_{C=N}$ thiadiazole), 1588($\nu_{C=N}$ imidazole), 1279(ν_{N-C}) ,768 (ν_{C-S}), 542 (ν_{C-Br}), 1490 ($\nu_{C=C}$), 685 (ν_{C-S-C} phenothiazine). ^{1}H NMR: δ(ppm) 6.73(1H, s, imidazole), 7.37-7.77 (4H, m, Ar-H aromatic ring),7.11-7.48 (8H, m Ar-H phenothiazine), ^{13}C NMR : δ (ppm) 120.7-132.1 (C of aromatic ring),124.5,145.5 and 116.5-128.3(C of phenothiazine),156.2,164.5(C_2,C_5 thiadiazole), 100.4 and 150.1 (C of imidazole), Anal. Calcd. for $C_{22}\ H_{13}\ Br\ N_4\ S_2$, C, 55.35, H, 2.74, N,11.74%, found C, 55.20, H, 2.52, N, 11.62%, MS 475.96 (M^+).

10-[2-(3-Bromo-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3e): M.P. 213-214°C, Yield 69%, IR:(ν max cm^{-1})1480(ν_{C-C}), 3174 (ν_{C-H}), 1638 ($\nu_{C=N}$ thiadiazole), 1590($\nu_{C=N}$ imidazole),1281(ν_{N-C})766 (ν_{C-S}), 540 (ν_{C-Br}) 1491 ($\nu_{C=C}$), 683(ν_{C-S-C} phenothiazine). ^{1}H NMR: δ(ppm) 6.76 (1H, s, imidazole), 7.41-7.61 (3H, m,Ar-H aromatic ring), 7.10-7.49 (8H, m, Ar-H phenothiazine), 7.76 (1H, td, J = 1.5, Hz, Ar-H aromatic ring),^{13}C NMR :δ (ppm)118.7-133.0 (C of aromatic ring), 124.6,145.7 and 116.7-128.4 (C of phenothiazine) , 175.1,164.4 (C_2,C_5 thiadiazole), 100.1 and 150.2(C of imidazole), Anal. Calcd. for $C_{22}\ H_{13}\ Br\ N_4\ S_2$: C, 55.35%, H, 2.74, N,11.74 found C, 55.20, H, 2.52, N, 11.62%; MS 475.98(M^+).

10-[2-(4-Bromo-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3f): M.P. 215-216°C, Yield 68 %, IR:(νmaxcm^{-1})1482 (ν_{C-C}), 3171 (ν_{C-H}), 1636 ($\nu_{C=N}$ thiadiazole), 1594 ($\nu_{C=N}$ imidazole), 1286 (ν_{N-C}) 769 (ν_{C-S}), 538 (ν_{C-Br}) 1489 ($\nu_{C=C}$), 688 (ν_{C-S-C} phenothiazine), ^{1}H NMR: δ(ppm) 6.72 (1H, s, imidazole), 7.69-7.78 (4H, m, Ar-H aromatic ring), 7.10-7.49 (8H, m Ar-H penothiazine), ^{13}C NMR :δ (ppm)- 124-131(C of aromatic ring), 124.2,145.8 and 116.8-128.7 (C of phenothiazine), 175.5,164.6 (C_2,C_5 thiadiazole), 100.3 and 150.7 (C of imidazole), Anal. Calcd. for $C_{22}H_{13}BrN_4S_2$, C, 55.35, H, 2.74, N, 11.74% found C, 55.19, H, 2.42, N, 11.72% ;MS 475.99 (M^+).

10-[2-(2-Nitro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine(3g): M.P. 215-217°C, Yield 74%, IR :(νmaxcm^{-1}) 1487 (ν_{C-C}), 3170 (ν_{C-H}),1642($\nu_{C=N}$ thiadiazole), 1598 ($\nu_{C=N}$ imidazole), 1287 (ν_{N-C}), 770 (ν_{C-S}), 1343 (ν_{C-NO2}) 1494 ($\nu_{C=C}$), 689 (ν_{C-S-C} phenothiazine), ^{1}H NMR: δ (ppm) 6.73 (1H, s, imidazole), 7.49-8.35 (4H, m, Ar-H aromatic ring), 7.11-7.48 (8H, m Ar-H phenothiazine), ^{13}C NMR : δ (ppm) 127.6-148.4 (C of aromatic ring), 124.7,144.9 and 115.9-127.8(C of phenothiazine), 156.5,164.4 (C_2,C_5 thiadiazole), 100.2 and 150.6 (C of imidazole), Anal. Calcd. for $C_{22}H_{13}N_5O_2S_2$, C, 59.58, H, 2.95, N, 15.79%, found C, 59.38, H, 2.85, N,15.59 %; MS 443.05 (M^+).

10-[2-(3-Nitro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3h): M.P. 216-218°C, Yield 75 %,IR: (vmax cm^{-1})(ν_{C-C}) 1483, (ν_{C-H}) 3172, 1641 ($\nu_{C=N}$ thiadiazole), 1596 ($\nu_{C=N}$ imidazole), 1286 (ν_{N-C}), 773 (ν_{C-S}), 1340 (ν_{C-NO2}) ,1492 ($\nu_{C=C}$), 685 (ν_{C-S-C} phenothiazine), ^{1}H NMR: δ (ppm) 6.68 (1H, s, imidazole), 7.58-8.84 (4H, m, Ar-H aromatic ring), 7.10-7.49 (8H, m Ar-H phenothiazine), ^{13}C NMR : δ (ppm) 116.4-140.5(C of aromatic ring), 124.8, 145.9 and 115.8-128.5(C of phenothiazine), 175.7,164.6 (C_2,C_5 thiadiazole), 100.1 and 150.9 C of imidazole, Anal. Calcd. for $C_{22}H_{13}N_5O_2S_2$,: C, 59.58 , H, 2.95, N, 15.79 found C, 59.40, H, 2.81, N, 15.55; MS 443.04 (M^+).

10-[2-(4-Nitro-phenyl)-imidazo[2,1-b][1,3,4]thiadiazol-6-yl]-10H-phenothiazine (3i): M.P.220-222°C,Yield 76%, IR: (vmax cm^{-1}) 1480 (ν_{C-C}), 3170 (ν_{C-H}), 1638 ($\nu_{C=N}$ thiadiazole), 1592 ($\nu_{C=N}$ imidazole), 1285 (ν_{N-C}), 770 (ν_{C-S}),1338 (ν_{C-NO2}), 1491 ($\nu_{C=C}$), 685 (ν_{C-S-C} phenothiazine), ^{1}H NMR: δ (ppm) 6.72 (1H, s, imidazole), 7.80-8.33 (4H, m, Ar-H aromatic ring), 7.11-7.49 (8H, m Ar-H phenothiazine), ^{13}C NMR : δ(ppm) 117.3-140.5(C of aromatic ring), 124.9,145.6 and 116.8-128 (C of phenothiazine),175.1,163.4 (C_2,C_5 thiadiazole),100.5 and 150.8 (C of imidazole), Anal. Calcd. for $C_{22}H_{13}N_5O_2S_2$,: C, 59.58, H, 2.95, N,15.79%, found C, 59.37, H, 2.81, N, 15.59%; MS 443.02 (M^+).

RESULTS AND DISCUSSION

The reaction of thiosemicarbazide and benzoic acid afforded compound **1**. The IR spectrum (ν cm^{-1}) of compound **1** showed absorption peaks at 1560-1650 for ($\nu_{N=C}$), 760-790 for ($\nu_{C-S,}$ thiadiaole ring) and peaks at 3350-3430 for (ν_{-NH2}). The ^{1}H NMR spectrum δ (ppm) exhibited the value at 3.9-4.9 (2H, s, NH_2), 7.28-7.95 (5H, m, Ar-H) and ^{13}C NMR spectrum gave δ (ppm) at 163-170 (C_2,C_5 of thiadiazole ring) supporting the structure of the compound **1**. Similarly the compounds **1** and **1a–1i** have been synthesized by taking the various derivatives of benzoic acid. The IR spectra gave absorptions in the range of 1665–1675 cm^{-1} while strong signals appeared in the range of δ (ppm) 3.9-4.9 and 7.39-8.20 in the ^{1}H NMR and δ(ppm)163-170 in the ^{13}C NMR spectra supported the formation of compounds **1a–1i** respectively. Another reaction was carried out between phenothiazine and chloroacetylchloride to give compound **2**. The IR spectrum showed absorptions at 1671 cm^{-1} due to the presence of carbonyl function, 685 (ν_{C-S-C} phenothiazine), 735(ν_{C-Cl}), 2836 (ν_{-CH2}). In the ^{1}H NMR, strong signals found at 4.35 (2H, s acyclic CH_2), 7.14-7.40 (8H, m,Ar-H)and ^{13}C NMR spectra gave signals at δ(ppm) 123.1-138.8 (C of phenothiazine ring), 165.5 (C=O acyclic), 42.2 (CH_2 acyclic) supporting the confirmation of synthesis of compound **2**. The compounds **1** and **1a–1i** on reaction with equimolar amount of compound **2** in ethanol gives the compounds **3** and **3a–3**i. These compounds showed a characteristic IR absorptions in the range of 1580–1600 cm^{-1} in the IR spectra showing the presence of N=C in the imidazole ring. The ^{1}H NMR spectra clearly indicated the presence of one proton in imidazole ring in the range of δ(ppm) 6.68–6.79. The ^{13}C NMR spectra of compound **3** and **3(a–i)** also supported the formation of imidazole ring, δ(ppm) 100.1– 100.9 and 149.9-150.9 the above structures were supported by fact that the disappearance of NH_2 proton and the appearance of N=CH proton in the range of δ(ppm) 6.68-6.79 (cyclic CH) in the ^{1}H NMR spectra of compound **3** and **3a–3i**. The compounds **2** and **2a–2i** and **3** and **3a–3i** were synthesized and compounds **3** and **3a–3i** screened for their antibacterial and antifungal activity against some selected bacteria and fungi respectively. Nitro group containing compounds showed higher activity in the order **(3i> 3g > 3h)** than chloro **(3c>3a >3b)** or bromo group containing compounds in the order **(3f> 3fd>3e)**. On the basis of structural activity relationship (SAR), concluded that the activity of compounds depends on electron withdrawing nature of the substituted groups, NO_2 > Cl > Br >H. The MIC value of the synthesized compounds and standard drug showed in the Table 2.

Biological Activity: All the newly synthesized compounds were active against selected microorganisms. The minimal inhibition diameters were determined by using the filter paper disc diffusion method [22-23] and the concentrations have been used in ppm, the compounds **3** and **(3a–3i)** have been screened in vitro for their antibacterial activity against B. *subtilis*, K. *pneumoniae* and S. *aureus* and antifungal activity against A. *niger*, A. *flavus* and C. *albicans*. Standards for antibacterial and antifungal activity streptomycin and griseofulvin were used respectively for comparison.

Physicochemical data of the synthesized compounds shown in table 1 and MIC value of the compounds compare in table 2.

Table 1: Characterisation data of the synthesized compounds 3 and 3(a-i)

Comp.	Ar1	Yield %	M.P. (^{0}C)	Molecular Formulae	MS (M^+)
3	C_6H_5	70	210-212	$C_{22}H_{14}N_4S_2$	398.06
3a	2-ClC_6H_4	71	209-211	$C_{22}H_{13}ClN_4S_2$	432.03
3b	3-ClC_6H_4	72	210-211	$C_{22}H_{13}ClN_4S_2$	432.02
3c	4-ClC_6H_4	70	212-214	$C_{22}H_{13}ClN_4S_2$	432.05
3d	2-BrC_6H_4	70	211-212	$C_{22}H_{13}BrN_4S_2$	475.96
3e	3-BrC_6H_4	69	213-214	$C_{22}H_{13}BrN_4S_2$	475.98
3f	4-BrC_6H_4	68	215-216	$C_{22}H_{13}BrN_4S_2$	475.99
3g	2-$NO_2C_6H_4$	74	215-217	$C_{22}H_{13}N_5O_2S_2$	443.05
3h	3-$NO_2C_6H_4$	75	216-218	$C_{22}H_{13}N_5O_2S_2$	443.04
3i	4-$NO_2C_6H_4$	76	220-222	$C_{22}H_{13}N_5O_2S_2$	443.02

Table 2. Antibacterial and antifungal activities of compounds 3 and 3(a–i).
Minimum Inhibition Concentration (MIC) given in µg mL^{-1}.

Comp.	B. *subtilis*	S. *aureus*	K. *pneumoniae*	A. *niger*	A. *flavus*	C. *albicans*
3	14	13	10	26.00	28.00	30.00
3a	4.00	3.15	3.50	12.25	13.60	12.65
3b	4.25	3.95	4.00	12.40	14.10	13.00
3c	3.78	3.10	3.30	12.00	13.20	12.30
3d	4.55	4.75	4.75	13.25	15.25	14.25
3e	5.10	5.45	5.00	13.75	15.50	15.75
3f	4.85	4.53	4.50	13.10	14.50	14.00
3g	2.12	2.75	3.35	10.45	10.95	10.80
3h	2.25	2.95	3.50	10.90	11.75	11.25
3i	2.10	2.30	2.40	9.30	11.00	10.50

The MIC values of standard streptomycin for all bacterial strain and griseofulvin for all fungi strain were in the range of 1.25–4.25 and 7.5–14.5 µg mL^{-1}, respectively

Table 3. Antibacterial activity (Inhibition Zone diameter in m.m.) of compounds 3 and 3 (a–i)

Comp.	B. *subtilis*		S. *aureus*		K. *pneumoniae*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3	4	7	6	8	3	5
3a	13.3	15	13.8	15.2	13.2	15.4
3b	13.2	14.5	13.2	14.8	13.0	15.1
3c	13.5	16	13.9	16	13.8	16
3d	12.6	13.2	12.	14.6	11.8	14.9
3e	12.1	12.9	11.8	14.2	11.2	14.2
3f	12.8	13.8	12.6	14.8	12.2	15.1
3g	16.5	21.3	17.0	22.5	17.5	23.9
3h	16.0	21.0	16.8	21.0	17.0	22.4
3i	17.2	21.9	17.5	22.9	17.9	24.5
SM*	19	25	18	24	20	26

Anti microbial activities: The synthesized compounds **3** and **3a–3i** were evaluated *in vitro* for antibacterial activity by using filter paper disc diffusion method against different strains of bacteria viz. B. *substilis,* E. *coli* and S. *tyhpi*. The entire final product along with standard antibacterial Streptomycin was used at 50 and 100 ppm concentrations. Antifungal activity against *A.niger, A. flavus* and *F. oxisporium* at 50 and 100 mg mL^{-1} concentrations by filter paper disc technique. The minimum inhibitory concentration (MIC) values of the synthesized compounds were determined.

Standard antibacterial streptomycin and antifungal griseofulvin were also tested under the similar conditions for comparison (Table 3 and 4).

Table 4. Antifungal activity(Inhibition Zone diameter in mm.) of compounds 3 and 3 (a–i)

Comp.	A. *niger*		A. *flavus*		C. *albicans*	
	50 ppm	100 ppm	50 ppm	100 ppm	50 ppm	100 ppm
3	8	10	7	9	10	13
3a	13.0	15.2	13.0	16.2	13.0	16.0
3b	12.8	14.5	12.6	15.4	12.8	15.5
3c	13.8	15.8	13.2	16.6	13.3	16.5
3d	12.4	13.0	12.2	14.0	12.1	14.2
3e	12.1	12.2	11.2	13.4	11.4	13.9
3f	12.2	13.2	12.9	14.2	12.8	15.0
3g	16.5	21.5	17.2	23.5	18.5	24.9
3h	16.0	21.1	16.2	22.9	17.4	23.4
3i	16.9	22.9	18.5	24.6	18.9	25.5
GF*	18	24	20	26	21	27

APPLICATION

The hybrid molecules we report are highly active. This novel series of compounds pave a way into a new class of antimicrobial compounds. There structures are flexible there are various reacting sites, which can be modified through structural optimizations to further enhance their activity and produce compounds which could result in main leads for new antimicrobial drugs. As we succeeded in our attempt of synthesizing hybrid substituted amino thiadiazole and chloro carbonyl derivative of phenothiazine and got highly encouraging results, there for medicinal chemists should focus on further development of such hybrids. In present work we focused on antimicrobial potential of these molecules. In conclusion our work has opened a new class of antimicrobial leads for medicinal chemists to work with.

CONCLUSIONS

In the conclusion we were successful in the initial hypothesis of synthesizing broad spectrum antibiotics through experimentation. We report a successful effort to combine pharmacophoric groups; 5-Phenyl-[1,3,4]thiadiazol-2-ylamine and Chloro-1-phenothiazin-10-yl-ethanone and the compounds were synthesised in good yield. The structures of compounds were established by FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry techniques. The synthesized compounds posses' broad spectrum activity against bacterial and fungal strains.

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