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Synthesis, spectral studies and biological activity of some novel biphenyl imidazo[2,1-b][1,3,4]thiadiazole derivatives

Amandeep Kaur*, Rajinder Kumar, Uday Kalidhar

Department of Pharmaceutical chemistry, ASBASJSM college of pharmacy, Bela (Ropar) 140111, Punjab, India

ABSTRACT

In the present study, we have reported the synthesis of some novel heterocyclic derivatives comprising imidazole and 1,3,4-thiadiazole containing biphenyl moiety. Imidazothiadiazoles are of interest because of their diverse biological activities and clinical applications. Reactions of biphenyl carboxylic acid with thiosemicarbazide in the presence of phosphorous oxychloride resulted in biphenyl containing 2-amino-1,3,4-thiadiazole which is then further subjected to condensation with α-bromoarylketone under reflux in dry ethanol. Synthesized novel derivatives of imidazo[2,1-b][1,3,4]thiadiazole containing biphenyl moiety. The structures of the compounds (V a-h and VIII a-h) were elucidated by spectral studies and screened for antibacterial activity against various strains of Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis, and antifungal activity against Candida albicans, Saccharomyces cerevisiae and Aspergillus niger. The derivatives has shown moderate to good activity when compared with standard antibiotic Ampicillin and Amphotericin B. And the two derivatives were screened for anti-cancer activity to National Cancer Institute (NCI), USA.

Keywords: Imidazo[2,1-b][1,3,4]thiadiazole; Biphenyl-4-carboxylic acid; 2-fluoro-biphenyl-4-carboxylic acid; Antimicrobial activity and anticancer activity.

***Corresponding author**
Email: tinasaini30@gmail.com

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INTRODUCTION

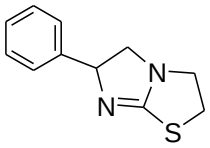
The fusion of a imidazole ring with a [1,3,4]thiadiazole nucleus give rise to a class of heterocyclic systems containing a bridgehead nitrogen atom known as imidazothiadiazoles. The structures of imidazo[2,1-b] [1,3,4]thiadiazoles are closely related to the biologically vibrant imidazo[1,3,4]thiazole heterocycles, in which the CH group in the thiazole ring is substituted by the isosteric nitrogen atom, but their properties often possess marked differences. The practically planar and rigid heteroaromatic imidazo[2,1-b][1,3,4] thiadiazole ring system may therefore have interesting physicochemical and biological properties, because of the presence of four heteroatoms and two condensed heterocycles with different π-conjugation[1].

The treatment of infectious diseases still remains an important and challenging problem because of a combination factors including emerging infectious diseases and increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria[2-7]. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistant bacterial strains in the last decades constitutes a substantial need for the new class of antibacterial agents[2,8]. Imidazole[2,1-b][1,3,4] thiadiazole derivatives have been of interest to the medicinal chemists for many years because of their anticancer[9], antitubercular[10], antibacterial[11], antifungal [12], anticonvulsant, analgesic [13] and antisecretory[14] activities. This is due to the fact that the imidazole [2,1-b][1,3,4] thiadiazole system is similar in part to Levamisole, a well-known immune modulator [15]. Levamisole (**1**) appears to be the most effective in patients with small tumor burdens and it acts by stimulating the responsiveness of lymphocytes to tumor antigens [16]. Anticancer drugs either kill cancer cells or modify their growth. Cancer or neoplastic disease, may be regarded as a family of related disorders. A common feature in different forms of cancer is an abnormal and uncontrolled cell division, frequently at a rate greater than that of most normal body cells. The neoplasm may be benign or malignant. Bening tumours do not metastasise, malignant tumours do. Metastasis is due to ability of neoplastic diseases to invade other tissues if a malignant cell floats away in the body fluids and locates in a distant place of the organism. So there occurs a secondary growth originating from the primary tumour[17].

The imidazo[2,1-b][1,3,4]thiadiazole ring system is the core skeleton of well known immunomodulator levamisole[18]. The anti-tumor potential of the 2-amino-1,3,4-thiadiazole skeleton was recognized in the early 1950's and subsequently its fusion with the imidazo[2,1-b] ring system has resulted in compounds with potential anti-cancer, analgesic, antibacterial, antisecretary and cytotoxic activities. Thiadiazole and its derivatives are used for biological activities such as antimicrobial, antitubercular, anti-inflammatory, anticonvulsant, antihypertensive, and anticancer[19].

We reported here a study on synthesis of some novel biphenyl imidazo[2,1-b][1,3,4]thiadiazole derivatives (V a-h and VIII a-h). These derivatives were screened for antibacterial activity against various strains of Escherichia coli, Pseudomonas aeruginosa and

Bacillus subtilis, and antifungal activity against Candida albicans, Saccharomyces cerevisiae and Aspergillus niger and were screened for anticancer activity.



Levamisole

MATERIALS AND METHODS

Chemistry

Most of the solvents used were of LR grade and were purified before use in different reactions. Chemicals used were of LR grade and obtained from Merck India, Loba chem., Central Drug House ltd, S. D. Fine chemicals ltd. and Alpha aesar etc. The solvents used throughout the experiment for running TLC were chloroform and methanol in the ratio of 9:1 and 95:05; toluene, ethyl acetate and formic acid in ratio of 5:4:1 (T:E:F) and benzene and ethanol in the ratio of 7:3. Iodine chambers were used for visualization of TLC spots. Melting points were determined on an electrothermal capillary melting point apparatus and were uncorrected. All the Infra red (IR) spectra were recorded by KBr pellets technique using Perkin Elmer IR spectrophotometer 4000-400 (ν_{max} in cm^{-1}). Proton magnetic resonance (^1H NMR) spectra were recorded on Bruker Advance II 400 (400 MHz) NMR spectrometer (chemical shift in ppm) in $CDCl_3$ and DMSO using Tetramethylsilane (TMS) as standard. Mass spectra of the synthesized compounds were recorded in MAT 120.

Synthesis of the compounds

Various **imidazo[2,1-b][1,3,4]thiadiazole** derivatives of **2-fluorobiphenyl-4-carboxylic acid and biphenyl-4-carboxylic acid** derivatives were synthesized using reaction **scheme 1 and scheme 2 respectively.**

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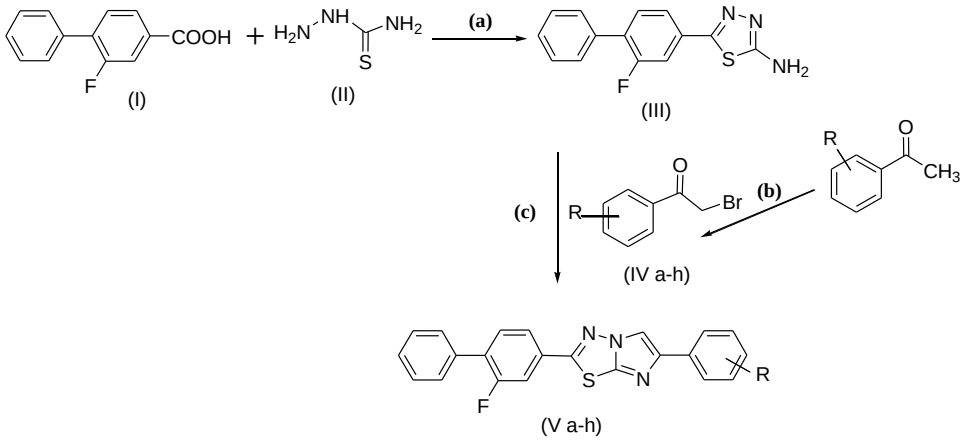
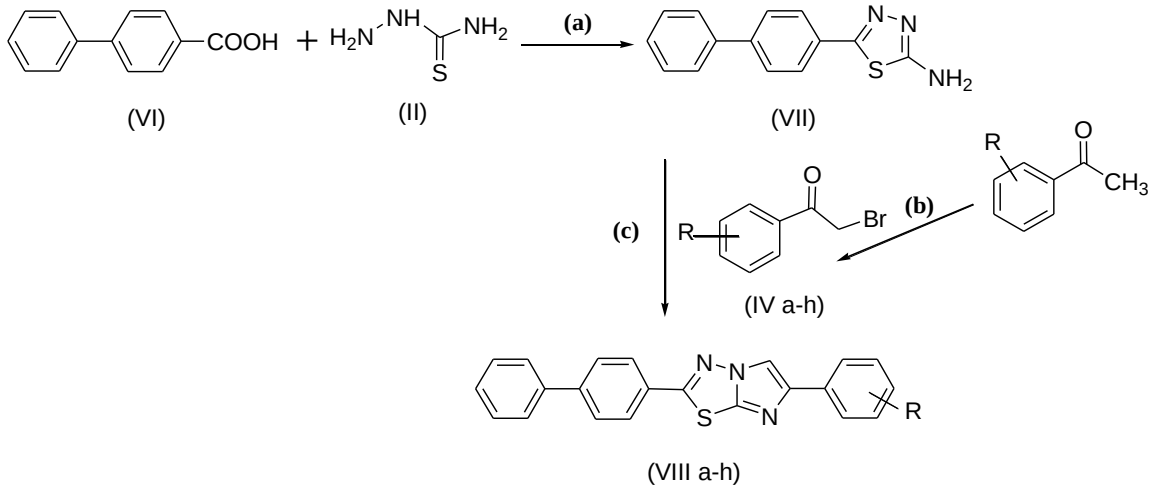
Scheme 1



Reactants: **a** = $POCl_3$; **b** = Br_2, Chloroform; **c** = Ethanol
R= **a**-H, **b**-4'Cl, **c**-4'F, **d**-2',4'diCl, **e**-4'NH_2, **f**-2',4'diOH, **g**-4'Br, **h**-2'OH

Scheme 2



Reactants: **a** = $POCl_3$; **b** = Br_2, Chloroform; **c** = Ethanol
R= **a**-H, **b**-4'Cl, **c**-4'F, **d**-2',4'diCl, **e**-4'NH_2, **f**-2',4'diOH, **g**-4'Br, **h**-2'OH

Synthesis of 5-(3-fluorobiphenyl-4-yl)-1,3,4-thiadiazol-2-amine (III) General procedure: 2-fluorobiphenyl-4-carboxylic acid (0.05 mol) (I) was refluxed with thiosemicarbazide (0.05 mol) (II) and phosphorus oxychloride (15 ml) for 45 min. The mixture was cooled and diluted with water (90 ml). And again refluxd for 4 hrs. on burner. Then the mixture was filtered and filtrate was basified with potassium hydroxide solution. The precipitate was filtered off and recrystallized from ethanol[20].

Synthesis of 5-(biphenyl-4-yl)-1,3,4-thiadiazol-2-amine (VII) General procedure: Biphenyl-4-carboxylic acid (0.05 M) (VI) was refluxed with thiosemicarbazide (0.05 M) (II) and phosphorus oxychloride (15 ml) for 45 min. The mixture was cooled and diluted with water (30 ml). And

again refluxd for 4 hrs. on burner. Then the mixture was filtered and filtrate was basified with potassium hydroxide solution. The precipitate was filtered off and recrystallized from ethanol[20].

Synthesis of Substituted Phenacyl Bromides (IV a-h) General procedure: Acetophenone (0.25 mol) was dissolved in 30 ml of chloroform. To the mixture bromine (0.25 mol) was added very slowly from dropping funnel with continuous stirring. During the addition the temperature was maintained below 20 ˚C. When the addition is complete, then cooled the mixture[21].

Synthesis of 2-(3-fluorobiphenyl-4-yl)-6-substituted[2,1-b][1,3,4]thiadiazole (V a-h) General procedure: A mixture of equimolar quantities (III) 5-(2-fluorobiphenyl-4-yl)-1,3,4-thiadiazol-2-amine (0.01 mol) and bromoacetyl compound (IV a-h) (0.01 mol) was refluxed in dry ethanol for 12 hrs. The excess of solvent was distilled off and the solid hydrobromide that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base. It was filtered, washed with water and dried[20].

Synthesis of 2-(biphenyl-4-yl)-6-substituted[2,1-b][1,3,4]thiadiazole (VIII a-h) General procedure: A mixture of equimolar quantities (VII) 5-(biphenyl-4-yl)-1,3,4-thiadiazol-2-amine (0.01 mol) and bromoacetyl compound (IV a-h) (0.01 mol) was refluxed in dry ethanol and dimethyl formamide for 12 hrs. The excess of solvent was distilled off and the solid hydrobromide that separated was collected by filtration, washed with water and dried[20].

5-(3-fluorobiphenyl-4-yl)-1,3,4-thiadiazol-2-amine (III): Yield 85%; m.p. 278-280 $^{\circ}$C; IR (KBr) cm^{-1}: 2964 (CH), 1579 (C=C), 3278 (N-H), 1072 (C-F); ^{1}H NMR (TMS) δ ppm: 7.36-7.82 (m, 8H, Ar-H), 3.50 (br s, 2H, NH_2).

2-(3-fluorobiphenyl-4-yl)-6-phenylimidazo[2,1b][1,3,4]thiadiazole (V-a): Yield 85%; m.p. 242-245 $^{\circ}$C; IR (KBr) cm^{-1}: 3057 (CH), 1580 (C=C), 1118 (C-F); ^{1}H NMR (TMS) δ ppm: 8.67 (s, 1H, C_5-H), 7.29-8.21 (m, 13H, Ar-H); ^{13}C NMR (TMS) δ ppm: 164.75, 145.98, 142.24, 141.71, 138.97, 133.91, 132.65, 132.14, 129.97, 128.68, 127.17, 126.94, 126.90, 122.43, 120.28, 119.00, 114.49; MS m/z: 272.1 (M^+), 273.1 (M+1), 274.1 (M+2).

6-(4-chlorophenyl)-2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazole (V-b): Yield 75%; m.p. 248-250 $^{\circ}$C; IR (KBr) cm^{-1}: 3128 (CH), 1580 (C=C), 1092 (C-F), 734 (C-Cl); ^{1}H NMR (TMS) δ ppm: 8.50 (s, 1H, C_5-H), 7.39-7.89 (m, 12H, Ar-H).

2-(3-fluorobiphenyl-4-yl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (V-c): Yield 78%; m.p. 253-258 $^{\circ}$C; IR (KBr) cm^{-1}: 3138 (CH), 1570 (C=C), 1108 (C-F); ^{1}H NMR (TMS) δ ppm: 8.45 (s, 1H, C_5-H), 7.36-7.88 (m, 12H, Ar-H).

6-(2,4-dichlorophenyl)-2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazole (V-d): Yield 71%; m.p. 261-264 $^{\circ}$C; IR (KBr) cm^{-1}: 3094 (CH), 1583 (C=C), 725 (C-Cl); ^{1}H NMR (TMS) δ ppm: 8.20 (s, 1H, C_5-H), 7.37-7.79 (m, 11H, Ar-H).

4-(2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (V-e): Yield 80%; m.p. 243-246 °C; IR (KBr) cm^{-1}: 3120 (CH), 1580 (C=C), 3246 (N-H); ^1H NMR (TMS) δ ppm: 8.45 (s, 1H, C_5-H), 7.39-8.01 (m, 12H, Ar-H), 3.50 (br s, 2H, NH_2).

4-(2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzene-1,3-diol (V-f): Yield 73%; m.p. 252-256 °C; IR (KBr) cm^{-1}: 3125 (CH), 1575 (C=C), 3357 (O-H); ^1H NMR (TMS) δ ppm: 8.39 (s, 1H, C_5-H), 7.35-7.86 (m, 11H, Ar-H), 5.30 (s, 2H, OH).

6-(4-bromophenyl)-2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazole (V-g): Yield 72%; m.p. 245-247 °C; IR (KBr) cm^{-1}: 3089 (CH), 1580 (C=C), 885 (C-Br); ^1H NMR (TMS) δ ppm: 8.45 (s, 1H, C_5-H), 7.36-7.86 (m, 12H, Ar-H).

2-(2-(3-fluorobiphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenol (V-h): Yield 77%; m.p. 267-270 °C; IR (KBr) cm^{-1}: 3089 (CH), 1575 (C=C), 3367 (O-H); ^1H NMR (TMS) δ ppm: 8.40 (s, 1H, C_5-H), 7.37-7.68 (m, 12H, Ar-H), 5.31 (s, 1H, OH).

5-(biphenyl-4-yl)-1,3,4-thiadiazol-2-amine (VII): Yield 80%; m.p. 289-295 °C; IR (KBr) cm^{-1}: 3150 (CH), 1600 (C=C), 3283 (N-H); ^1H NMR (TMS) δ ppm: 7.27-8.07 (m, 9H, Ar-H), 3.37 (s, 2H, NH_2).

2-(biphenyl-4-yl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole (VIII-a): Yield 85%; m.p. 263-260 °C; IR (KBr) cm^{-1}: 3029 (CH), 1596 (C=C); ^1H NMR (TMS) δ ppm: 8.54 (s, 1H, C_5-H), 7.39-8.16 (m, 14H, Ar-H); ^{13}C NMR (TMS) δ ppm: 145.33, 13133.69, 133.57, 129.92, 128.60, 128.53, 127.92, 127.75, 127.37, 126.74, 126.61; MS m/z: 254.1 (M^+), 255.1 (M+1), 256.1 (M+2).

2-(biphenyl-4-yl)-6-(4-chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (VIII-b): Yield 76%; m.p. 271-274 °C; IR (KBr) cm^{-1}: 3055 (CH), 1595 (C=C), 725 (C-Cl); ^1H NMR (TMS) δ ppm: 8.50 (s, 1H, C_5-H), 7.28-7.58 (m, 13H, Ar-H).

2-(biphenyl-4-yl)-6-(4-fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (VIII-c): Yield 79%; m.p. 261-264 °C; IR (KBr) cm^{-1}: 3089 (CH), 1587 (C=C), 1150 (C-F); ^1H NMR (TMS) δ ppm: 8.45 (s, 1H, C_5-H), 7.25-7.68 (m, 13H, Ar-H).

2-(biphenyl-4-yl)-6-(2,4-dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazole (VIII-d): Yield 80%; m.p. 255-259 °C; IR (KBr) cm^{-1}: 3080 (CH), 1580 (C=C), 736 (C-Cl); ^1H NMR (TMS) δ ppm: 8.54 (s, 1H, C_5-H), 7.38-8.07 (m, 12H, Ar-H).

4-(2-(biphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)aniline (VIII-e): Yield 73%; m.p. 263-267 °C; IR (KBr) cm^{-1}: 3055 (CH), 1595 (C=C), 3255 (N-H); ^1H NMR (TMS) δ ppm: 8.51 (s, 1H, C_5-H), 7.36-8.01 (m, 13H, Ar-H), 3.35 (br s, 2H, NH_2).

4-(2-(biphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl) benzene-1,3-diol (VIII-f): Yield 75%; m.p. 266-269 °C; IR (KBr) cm^{-1}: 3057 (CH), 1589 (C=C), 3367 (O-H); ^{1}H NMR (TMS) δ ppm: 8.25 (s, 1H, C_5-H), 7.35-8.07 (m, 12H, Ar-H), 5.34 (s, 2H, OH).

2-(biphenyl-4-yl)-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazole (VIII-g): Yield 81%; m.p. 254-259 °C; IR (KBr) cm^{-1}: 3056 (CH), 1585 (C=C), 876 (C-Br); ^{1}H NMR (TMS) δ ppm: 8.28 (s, 1H, C_5-H), 7.28-7.99 (m, 13H, Ar-H).

2-(2-(biphenyl-4-yl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)phenol (VIII-h): Yield 79%; m.p. 253-257 °C; IR (KBr) cm^{-1}: 3089 (CH), 1580 (C=C), 3348 (O-H); ^{1}H NMR (TMS) δ ppm: 8.45 (s, 1H, C_5-H), 7.36-8.01 (m, 13H, Ar-H), 5.30 (s, 1H, OH).

Antimicrobial susceptibility test

The newly synthesized compounds were screened for their antibacterial and antifungal screening using agar diffusion method.

The antibacterial activity of test compounds were evaluated against Gram-positive bacteria, Bacillus subtilis and Gram-negative bacteria, Escherichia coli; Pseudomonas aeruginosa.

Antifungal activity was screened against three fungal strain, Candida albicans; Saccharomyces cerevisiae and Aspergillus niger. The bacterial cultures were inoculated on Nutrient Agar and fungal culture was inoculated on Potato Dextrose Agar.

The nutrient agar media was taken in a 1000ml beaker and made up the volume to 1000ml with water then the media was sterilized by autoclaving at 121 ˚C for 15 min at 15-psi pressure. Afterwards the mixture was cooled to 45 ˚C and then inoculums were added to the above cooled media, mixed properly and poured into the sterile petridishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test solution and standard solution at a concentration of 50 µg/ml were taken. The standard antibiotic (Ampicillin) for bacteria and (Amphotericin B) for fungal was maintained with same concentration in each plate and a control having only DMSO in one plate. Then petridishes were incubated at 37 ˚C for 24 hrs and zones of inhibition were observed and measured.

In vitro cancer screen at NCI-USA

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10 µM. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-1 glutamine. For a typical

screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 µM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$$[(Ti-Tz)/(C-Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$
$$[(Ti-Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz.$$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) is calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is

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reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested[22-24].

RESULTS AND DISCUSSION

The synthetic route of compounds (V a-h and VIII a-h) is outlined in Schemes 1 and 2, respectively. 2-Amino-5-alkyl/aryl-1,3,4-thiadiazole **1** was obtained by direct cyclisation of a alkyl/aryl moiety and thiosemicarbazide in the presence of phosphorus oxychloride, the latter refluxed with substituted α-haloaryl ketones in dry ethanol yielded the imidazothiadiazoles in good yield. 2-Amino-5-alkyl/aryl-1,3,4-thiadiazole **2** was obtained by direct cyclisation of a alkyl/aryl moiety and thiosemicarbazide in the presence of phosphorus oxychloride, the latter refluxed with substituted α-haloaryl ketones in dry ethanol yielded the imidazothiadiazoles in good yield.

All the compounds were confirmed by its IR spectrum, which showed the presence of particular functional groups. The absorption at 3128-3029 are characteristic of v(C-H) and v(C=C) respectively. The appearance of imidazole proton (C_5-H) around δ 8 and the aromatic proton signals showed δ (7.1-8.2) in the ^{1}H NMR spectra. The ^{13}C-NMR and Mass spectral data on synthesized compounds are also in accordance with the proposed structures. The physical data and the yield of the synthesized compounds are given in Table-1.

Table 1: Physical data of compounds (V a-h and VIII a-h)

Compounds	Yield (%)	m.p. (ºC)	Molecular Formula
V a	85	242-245	$C_{22}H_{14}FN_3S$
V b	75	248-250	$C_{22}H_{13}ClFN_3S$
V c	78	253-258	$C_{22}H_{13}F_2N_3S$
V d	71	261-264	$C_{22}H_{12}Cl_2FN_3S$
V e	80	243-246	$C_{22}H_{15}FN_4S$
V f	73	252-256	$C_{22}H_{14}FN_3O_2S$
V g	72	245-247	$C_{22}H_{13}BrFN_3S$
V h	77	267-270	$C_{22}H_{14}FN_3OS$
VIII a	85	263-266	$C_{22}H_{15}N_3S$
VIII b	76	271-274	$C_{22}H_{14}ClN_3S$
VIII c	79	261-264	$C_{22}H_{14}FN_3S$
VIII d	80	255-259	$C_{22}H_{13}Cl_2N_3S$
VIII e	73	263-267	$C_{22}H_{16}N_4S$
VIII f	75	266-269	$C_{22}H_{15}N_3O_2S$
VIII g	81	254-259	$C_{22}H_{14}BrN_3S$
VIII h	79	253-257	$C_{22}H_{15}N_3OS$

The imidazo[2,1-b][1,3,4]thiadiazole derivatives were assayed in vitro for their antimicrobial activity against a panel of selected Gram-positive, Gram-negative bacteria and fungi in Table-2, in comparison with those of the standard drugs ampicillin and amphotericin B. The antibacterial activity data reveals that the compounds (V a-h) and (VIII a-h) exhibited good antibacterial activity against various strains of bacteria as compared to standard Ampicillin.

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Table 2: Antibacterial and antifungal activities of compounds (V a-h and VIII a-h)

Compound Code	Zone of Inhibition (mm)					
	P.aeruginosa	B.subtilis	E.coli	C.albicans	S.cerevisiae	A. niger
V a	9.1	7.2	10.5	4.4	-	3.2
V b	8.5	8.5	9.4	5.8	-	4.5
V c	8.9	9.3	6.7	-	-	-
V d	7.9	9.8	8.8	-	-	-
V e	9.9	6.5	8.2	3.7	-	-
V f	9.5	7.8	7.8	-	-	2.7
V g	10.2	6.9	7.3	4.3	-	-
V h	9.6	9.4	9.7	-	-	-
VIII a	7.5	9.1	8.9	3.4	-	4.4
VIII b	8.5	8.5	9.4	4.8	-	-
VIII c	9.0	9.3	6.7	-	-	-
VIII d	7.9	7.4	9.4	-	-	-
VIII e	6.5	6.5	8.2	4.4	-	3.8
VIII f	9.5	7.8	8.7	-	-	-
VIII g	8.8	8.4	7.3	4.3	-	-
VIII h	9.6	6.9	9.7	-	-	-
STD	14.5	13.0	15.5	14.0	13.0	15.0

Control: DMSO; (-): No zone of inhibition

The antifungal screening results showed moderate activity against Candida albicans and Aspergillus niger strains as compared to standard Amphotericin B and no activity against Saccharomyces cerevisiae.

The tumor growth inhibition properties of the compound **VIII b & VIII d** with the **NCI code ID-105053 & ID-105054** selected by the National Cancer Institute (NCI), USA, were screened on 56 and 57 human tumor cell lines at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI in a primary one dose anti cancer assay.

Primary single high dose (10^5 M) full NCI 60 cell panel in vitro assay

All the selected compounds submitted to National Cancer Institute (NCI) for in vitro anticancer assay were evaluated for their anticancer activity. Primary in vitro one dose anticancer assay was performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon brain breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^5 M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B. The compound VIII b & VIII d showed moderate anticancer activity in table-3.

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Table 3: NCI in vitro testing result of compound VIII b & VIII d at single dose level in µM

Panel	Cell Line	GI_{50} (Concentration per cell line) VIII-b	VIII-d
Leukemia			
	HL-60(TB)	50.31	70.38
	K-562	97.00	105.31
	MOLT-4	78.69	99.34
	RPMI-8226	84.37	109.81
	SR	95.72	101.46
Non-Small Cell Lung Cancer			
	A549/ATCC	85.18	99.58
	EKVX	92.73	96.64
	HOP-62	5.27	108.79
	HOP-92	85.50	101.05
	NCI-H226	92.86	110.60
	NCI-H23	86.05	100.48
	NCI-H322M	92.99	98.99
	NCI-H460	89.00	112.33
	NCI-H522	82.77	82.93
Colon Cancer			
	COLO 205	103.70	128.37
	HCC-2998	101.97	104.85
	HCT-116	48.29	95.42
	HCT-15	91.02	87.85
	HT29	55.33	89.43
	KM12	90.27	114.12
	SW-620	108.16	115.47
CNS Cancer			
	SF-268	54.95	110.20
	SF-295	88.00	94.38
	SF-539	97.48	105.23
	SNB-19	65.38	106.11
	U251	59.11	99.37
Melanoma			
	LOX IMVI	80.05	106.58
	MALME-3M	93.01	98.59
	M14	98.74	102.44
	MDA-MB-435	84.34	97.40
	SK-MEL-28	103.66	101.80
	SK-MEL-5	99.45	104.89
	UACC-257	115.18	110.77
	UACC-62	83.10	99.91
Ovarian Cancer			
	IGROV1	92.40	103.55
	OVCAR-3	118.75	128.09
	OVCAR-4	84.81	110.91
	OVCAR-5	98.08	103.99
	OVCAR-8	27.01	111.24
	NCI/ADR-RES	55.21	108.67
	SK-OV-3	98.54	118.09

Renal Cancer			
	786-0	68.75	86.33
	A498	108.17	99.16
	ACHN	53.34	102.86
	CAKI-1	82.63	96.01
	RXF 393	62.57	112.92
	SN12C	75.61	100.12
	TK-10	99.87	88.88
	UO-31	87.28	98.02
Prostate Cancer			
	PC-3	85.89	97.01
	DU-145	113.03	110.50
Breast Cancer			
	MCF7	46.13	93.97
	MDA-MB-231/ATCC	98.27	120.75
	HS 578T	111.40	124.54
	BT-549	98.12	86.56
	T-47D	81.04	95.63
	MDA-MB-468	112.67	117.63

CONCLUSION

All the newly synthesized biphenyl imidazo[2,1-b][1,3,4]thiadiazole derivatives were analysed with different spectral techniques and screened in vitro for their antibacterial activity against both Gram-positive and Gram-negative strains of bacteria and also subjected for the antifungal activity. The results of antimicrobial screening reveals all compounds exhibited good activity against all strains and moderate activity against Candida albicans and Aspergillus niger strains and no activity against Saccharomyces cerevisiae strain. The newly synthesized compounds of series were also screened for anticancer activity to National Cancer Institute, USA.

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REFERENCES

[1] Khazi IAM, Gadad AK, Lamani RS, Bhongade BA. Tetrahedron 2011; 67: 3289-3316.
[2] Alagawadi KR, Alegaon SG. Arabian J Chem 2010: doi:10.1016/j.arabjc.2010.07.012.
[3] Tenover FC, McDonald LC. Curr Opin Infect Dis 2005; 18: 300-305.
[4] Pfeltz RF, Wilkinson BJ. Curr Drug Targets Infect Disord 2004; 4: 273-294.
[5] Roberts MC. Curr Drug Targets Infect Disord 2004; 4: 207-215.
[6] Dessen A, Di Guilmi AM, Vernet T, Dideberg O. Curr Drug Targets Infect Disord 2001; 1: 63-77.
[7] Muroi H, Nihei K, Tsujimoto K, Kubo I. Biorg Med Chem 2004; 12: 583-587.

[8] Chopra I, Schofield C, Everett M, O'Neill K, Miller K, Wilcox M. Lancet Infect Dis 2008; 8: 133-139.
[9] Zheng KB,He J, Zhang J. Chinese Chem Lett 2008; 19: 1281-1284.
[10] Gadad AK, Noolvi MN, Karpoormath RV. Bioorg Med Chem 2004; 12: 5651-5659.
[11] Gadad AK, Mahajanshetti CS, Nimbalkar S, Raichurkar A. Eur J Med Chem 2000; 35: 853-857.
[12] Andotra CS, Langer TC, Kotha A. J Indian Chem Soc 1997; 74(2): 125-127.
[13] Khazi IAM, Mahajanshetti CS, Gadad AK, Tarnalli AD, Sultanpur CM. Arzneim-Forsch./Drug Res 1996; 46: 949-952.
[14] Andreani A, Leonia A, Locatelli A, Morigi R, Rambaldi M, Simon WA, Senn-Bilfinger J. Arzneim-Forsch./Drug Res 2000; 50: 550-553.
[15] Banu A, Vasundhara DE, Lamani RS, Khazi IAM, Begum NS. J Saudi Chem Soc 2011 doi:10.1016/j.jscs.2011.03.010.
[16] Karki SS, Panjamurthy K, Kumar S, Nambiar M, Ramareddy SA, Chiruvella KK, Raghavan SC. Eur J Med Chem 2011; 46: 2109-2116.
[17] Kapoor VK. Medicinal and Pharmaceutical Chemistry. Vallabh Prakashan Delh. 2005; 2: 591.
[18] Terzioglu N, Gursoy A. Eur J Med Chem 2003; 38: 781-786.
[19] Kolavi G, Hegde V, Khazi IA, Gadad P. Bioorg Med Chem 2006; 14: 3069-3080.
[20] Gadad AK, Palkar MB, Anand K, Noolvi MN, Boreddy TS, Wagwade J. Bioorg Med Chem 2008; 16: 276-283.
[21] Lamani RS, Shetty NS, Kamble RR, Khazi IAM. Eur J Med Chem 2009; 44: 2828-2833.
[22] Alley MC, Scudiero DA, Monks PA, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Cancer Res 1988; 48: 589-601.
[23] Grever MR, Schepartz SA, Chabner BA. Semin Oncol 1992; 19: 622-638.
[24] Boyd MR, Paull KD. Drug Dev Res 1995; 19: 91-109.