Asian Journal of Chemistry; Vol. 27, No. 11 (2015), 4145-4152

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.19136

Synthesis, Characterization and Solvatochromic Effect of Some Azo Based 2-Thioxopyrimidine-4,-6-dione Analogues and their Antimicrobial Evaluation

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Received: 27 February 2015; *Accepted*: 15 April 2015; *Published online*: 16 July 2015; AJC-17422

Aim of the present study was to synthesize 5-aryl/heteroaryl azo pyrimidine analogues and to explore their antibacterial activity. A series of 5-aryl/heteroaryl azo bearing pyrimidine analogues (**4i-4xii**) were synthesized. The compounds were characterized by different modern analytical techniques. Solvatochromic behaviours of these compounds were characterized by UV-visible spectrophotometer. Most of the synthesized compounds possess significant biological activity. The antibiogram pattern revealed that compound 4-nitro phenyl azo substituted thiobarbituric acid (**4ii**) exhibited broad spectrum antimicrobial activity followed by compounds 4-bromo, 3-methyl, phenyl azo substituted (**4v**) and 2-methoxy phenyl azo substituted thiobarbituric acid (**4vii**). The compounds **4iv**, **4viii** and **4ix** exhibited moderate inhibition. However, compound **4iii** exhibited least zones of inhibition.

Keywords: Antibiogram, LC-MS, XRD, Exothermic, Pyrimidine, Solvatochromic.

INTRODUCTION

Blindly using of antibiotics increases the antibiotic resistance among pathogenic bacteria is a major calamity for clinical management of various infectious diseases[1]. Searching of new antibacterial agents excluding antibiotic analogues encourages many medicinal chemists[2]. At present urinary tract infections are very common due to various bacterial as well as fungal pathogens. More commonly it occurs in women. Physicians recommended a course of antibiotic for the treatment. Alternative to antibiotic is the only solution for such type of problems and the ultimate objective of this research work. 2-Thiobarbituric acid (TBA) is chemically 2-thioxopyrimidine-4,6-dione and synthetically cyclic malonyl thiourea derivative and was an intermediate synthetic precursor, from which various thiobarbiturates have been synthesized and reported to possess wide range pharmacological application such as, general anaesthesia, sedation, anticonvulsant and anxiolytic effect[3]. Thiobarbiturate derivatives also exhibited antibacterial[4], antifungal[5] and antioxidant[6] effect. As per structural activity relationship of 2-thiobarbituric acid, the arylidene moiety substituted at C-5 position may responsible for exhibiting CNS depressant and urease inhibitory action[7].

Azo bearing heterocyclic compounds are well recognized for their medicinal uses such as antibacterial[8], antifungal[9], antitumor[10] and antiseptic[11]. The azo linked dye sulfonamide prontosil was the first effective chemotherapeutic prodrug to be employed systemically for prevention and use of streptococcal infections in human[12]. Introduction of hydrazino and diazo group have been reported to enhance the pharmacological activities of heterocyclic compounds[13]. Pyrazolones are associated with broad spectrum biological activities including antibacterial activity[14]. Previously, our research has been reported that azo bearing thiobarbiturates act as potent antimicrobial agents[15]. Some of the effective biological active drugs containing azo linkage are darcarbazine (anti alkylating agent), sulfasalazine (antiulcerative agent) and balasalazide (anti-inflammatory agent). In continuation of the earlier reported work, this part of work is included with synthesis and biological evaluation of some unreported heteroaryl/aryl azo thiobarbiturates. Further, the solvatochromic behavior of newly synthesized thiobarbiturates are studied by UV and characterized by thermal and XRD analysis).

Keeping in view of the valuable information regarding the biological activities through literature survey, it was thought of interest to insert aryl/heteroaryl azo groups at C-5 position of 2-thiobarbituric acid by azocoupling reaction and to investigate their antimicrobial activity against different Gram-positive and Gram-negative bacterial strains.

EXPERIMENTAL

The chemicals used for present research studies were of synthetic grade and belongs to Merck Company Ltd. The

prepared products were analyzed by FT/IR (JASCO FT/IR 4100 Spectrophotometer using KBr disc), ^{1}H NMR (Bruker ^{1}H NMR 400 MHz) using TMS as an internal standard, LC-MS (Shimadzu-Mass spectrometer). X-ray diffraction (XRD) pattern of silica was obtained with CuK_α X-ray source and a step of 0.02 (2θ) and run 2θ = 6-80° at 37 °C and the Differential scanning calorimetry (DSC) measurement was made on the instrument METTLER TOLEDO STAR^e system at a heating rate of 10 °C/min in nitrogen atmosphere for the synthesized compound using aluminium cans calibrated with indium to ensure accuracy of the calorimetric scale. UV (JASCO V-630 Spectrophotometer) and elemental analysis was carried out by using Perkin Elmer-2400 CHNO/S analyzer system. The melting points were determined by open capillary method and uncorrected.

The observed data on zone of inhibition for different synthesized compounds on different microbial strains were subjected to one way-analysis of variance (ANOVA) for comparison of mean. The results of antimicrobial activity were statistically interpreted by Dunnett *t*-test. A sample size of three has been taken in the study for each compound against each strain and results are expressed as mean ± SD.

Synthesis of (E)-5-(substituted phenyl diazenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione[16]: A cold solution of 2.5 mL of sodium nitrite (0.207 g, 3 mmol) was added drop wise to ice-cold solution of twelve different individual substituted aromatic amines in conc. HCl and water in equal proportion. The temperature of the reaction was maintained within a range of 0-5 °C. The diazotized solutions poured into an ice cold solution of 2-thiobarbituric acid in 10 % (20 mL) sodium hydroxide solution. The coloured products obtained were filtered, washed with water and dried. Finally obtained products re-crystallized from ethanol. The progress of reaction was monitored by TLC using suitable solvent system.

4-[(4,6-Dioxo-2-thioxohexahydropyrimidin-5-yl)-diazenyl]benzenesulfonamide (4i): Brown colour powder; Yield 80 %; R_f: 0.7, m.p. (°C): 300-310; UV-visible ($\lambda_{max,}$ DMF): 409 nm; IR (KBr, ν_{max}, cm^{-1}) : 3419 (N-H str), 3091 (Ar-H), 1709 (C=O str), 1635 (C=N str), 1497 (-N=N-), 1354 (C=S str),1245 (C-O str), 1320, 1175 (SO_2 str), 905 (S-N str); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.25 (s, 1H, thiobarbituric acid NH), 11.85 (s, 1H, thiobarbituric acid NH), 14.55 (s, 1H, thiobarbituric acid OH), 7.83-7.91 (m, 4H, Ar-H), 7.39 (s, 2H, SO_2NH_2), 2.75 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.571, 100; *m/z*; 326.02 (M-1); Analysis calcd. (%) for $C_{10}H_9N_5O_4S_2$: C, 36.69; H, 2.77; N, 21.39; S, 19.59. Found (%): C, 36.67; H, 2.75; N, 21.36; S, 19.58.

5-[(4-Nitrophenyl)diazenyl]-2-thiobarbituric acid (4ii): Brown colour powder; Yield 80 %; R_f: 0.8, m.p. (°C): 295-305; UV-visible ($\lambda_{max,}$ DMF): 430 nm; IR (KBr, ν_{max}, cm^{-1}): 3438 (N-H str), 3096 (Ar-H), 1706, 1659 (C=O str), 1492 (-N=N-), 1343(C=S str), 1249 (C-O str), 1397, 1555 (NO_2 str); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.35 (s, 1H, thiobarbituric acid NH), 11.65 (s, 1H, thiobarbituric acid NH), 14.35 (s, 1H, thiobarbituric acid OH), 7.79-8.32 (m, 4H, Ar-H), 2.77 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.115, 100; *m/z*; 294.16 (M+1); Analysis calcd. (%) for $C_{10}H_7N_5O_4S$: C, 40.96; H, 2.41; N, 23.88; S, 10.93. Found (%): C, 40.97; H, 2.39; N, 23.87; S, 10.91.

4-[(4,6-Dioxo-2-thioxohexahydropyrimidin-5-yl)-diazenyl]benzenesulfonic acid (4iii): Yellow colour powder; Yield 65 %; R_f: 0.8, m.p. (°C): 290-300; UV-visible ($\lambda_{max,}$ DMF): 419 nm; IR (KBr, ν_{max}, cm^{-1}): 3450 (N-H str), 3020 (Ar-H), 1709, 1657 (C=O str), 1507 (-N=N-), 1354 (C=S str), 1254(C-O str), 1298, 1197 (SO_2 str); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.25 (s, 1H, thiobarbituric acid NH), 11.55 (s, 1H, thiobarbituric acid NH), 14.25 (s, 1H, thiobarbituric acid OH), 7.83-7.95 (m, 4H, Ar-H), 2.91 (s, 2H, SO_2OH), 2.85 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 0.915, 100; *m/z*; 326.93 (M-1); Analysis calcd. (%) for $C_{10}H_8N_4O_5S_2$: C, 36.58; H, 2.46; N, 17.06; S, 19.53. Found (%): C, 36.55; H, 2.44; N, 17.05; S, 19.51.

5-[(4-Methoxyphenyl)diazenyl]-2-thiobarbituric acid (4iv): Brick red colour powder; Yield 75 %; R_f: 0.7, m.p. (°C): 310-320; UV-visible ($\lambda_{max,}$ DMF): 438 nm; IR (KBr, ν_{max}, cm^{-1}): 3570 (N-H str), 2933 (aryl-OCH_3), 3020 (Ar-H), 1714 (C=O str), 1640 (C=N str), 1530 (-N=N-), 1346 (C=S str), 1250 (C-O str), 1290(=C-O-CH_3 asym str), 1129(=C-O-CH_3 symstr); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 7.56-7.68 (s, 1H, thiobarbituric acid NH), 6.82-7.26 (m, 4H, Ar-H), 3.83 (s, 3H,OCH_3), 2.71 (s, 1H, CH-N=N-); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.45 (s, 1H, thiobarbituric acid NH), 11.75 (s, 1H, thiobarbituric acid NH), 14.55 (s, 1H, thiobarbituric acid OH), 6.82-7.26 (m, 4H, Ar-H), 3.83 (s, 3H, OCH_3), 2.71 (s, 1H, CH-N=N-); LC-MS: 278.29 (M); Analysis calcd. (%) for $C_{11}H_{10}N_4O_3S$: C, 47.48; H, 3.62;N, 20.13; S, 11.52. Found (%): C, 47.45; H, 3.61; N, 20.11; S, 11.53.

5-[(4-Bromo-3-methylphenyl)diazenyl]-2-thiobarbituric acid (4v): Light yellow colour powder; Yield 75 %; R_f: 0.6, m.p. (°C): 320-330; UV-visible (λ_{max} DMF): 415 nm; IR (KBr, ν_{max}, cm^{-1}): 3575 (N-H str), 2956 (aryl-CH_3), 2994 (Ar-H), 1709 (C=O str), 1509 (-N=N-), 1355 (C=S str),1246 (C-O str), 716,746 (-C-Br bend); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.25 (s, 1H, thiobarbituric acid NH), 11.62 (s, 1H, thiobarbituric acid NH), 14.45 (s, 1H, thiobarbituric acid OH), 6.99-7.49 (m, 3H, Ar-H), 2.34 (s, 3H, CH_3), 2.50 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 2.105, 100; *m/z*; 338.9 (M-1); Analysis calcd. (%) for $C_{11}H_9BrN_4O_2S$: C, 38.72; H, 2.66; N, 16.42; S, 9.40. Found (%): C, 38.70; H, 2.63; N, 16.39; S, 9.37.

5-(Thiazol-2-yldiazenyl)-2-thiobarbituric acid (4vi): Grayish black colour powder; Yield 55 %; R_f: 0.8, m.p. (°C): 285-290; UV-visible ($\lambda_{max,}$ DMF): 416 nm; IR (KBr, ν_{max}, cm^{-1}): 3460 (N-H str), 2886 (heteroaryl-CH), 1702, 1664 (C=O str), 1494(-N=N-), 1322 (C=S str), 1212 (C-O str), 807,722 (heteroaryl -CH bend); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.65 (s, 1H, thiobarbituric acid NH), 11.95 (s, 1H, thiobarbituric acid NH), 12.50 (s, 1H, thiobarbituric acid OH), 7.43-7.67 (d, 2H, Thiazole-H), 2.50 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.561, 100; *m/z*; 253.95 (M-2); Analysis calcd. (%) for $C_7H_5N_5O_2S_2$: C, 32.93; H, 1.97; N, 27.43; S, 25.12. Found (%): C, 32.91; H, 1.95; N, 27.44; S, 25.10.

5-[(2-Methoxyphenyl)diazenyl]-2-thiobarbituric acid (4vii): Red colour powder; Yield 70 %; R_f: 0.6, m.p. (°C): 230-240; UV-visible ($\lambda_{max,}$ DMF): 432 nm; IR (KBr, ν_{max}, cm^{-1}): 3488 (N-Hstr), 2922 (aryl-OCH_3), 3020 (Ar-H),1694 (C=O

str), 1635 (C=N str), 1482(-N=N-), 1350 (C=S str), 1248(C-O str), 1310 (=C-O-CH_3 asymstr), 1149 (=C-O-CH_3 symstr); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.15 (s, 1H, thiobarbituric acid NH), 11.52 (s, 1H, thiobarbituric acid NH), 14.39 (s, 1H, thiobarbituric acid OH), 6. 75-7.34 (m, 4H, Ar-H), 3.83 (s, 3H, OCH_3), 2.97 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.765, 100; *m/z*; 277.13 (M-1); Analysis calcd. (%) for $C_{11}H_{10}N_4O_3S$: C, 47.48; H, 3.62; N, 20.13; S, 11.52. Found (%): C, 47.46; H, 3.60; N, 20. 11; S, 11.53.

5-[(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl]-2-thiobarbituric acid (4viii): Orange colour powder; Yield 62 %; R_f: 0.8, m.p. (°C): 250-260; UV-visible ($\lambda_{max,}$ DMF): 426 nm; IR (KBr, ν_{max}, cm^{-1}): 3452 (N-H str), 2883 (heteroaryl -CH), 1655 (C=O str), 1516 (-N=N-), 1325 (C=S str),1254 (C-O str), 771 (heteroaryl -CH bend); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.25 (s, 1H, thiobarbituric acid NH), 11.52 (s, 1H, thiobarbituric acid NH), 14.29 (s, 1H, thiobarbituric acid OH), 7.26-7.45 (m, 5H, Ar-H), 3.14 (s, 3H, N-CH_3), 2.17 (s, 3H,C-CH_3), 3.40 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.685, 100; *m/z*; 359.13 (M+1); Analysis calcd. (%) for $C_{15}H_{14}N_6O_3S$: C, 50.27; H, 3.94; N, 23.45; S, 8.95. Found (%): C, 50.25; H, 3.91; N, 23.42; S, 8.93.

5-[(3-Nitrophenyl)diazenyl]-2-thiobarbituric acid (4ix): Greenish yellow colour powder; Yield 80 %; R_f: 0.7, m.p. (°C): 280-290; UV-visible ($\lambda_{max,}$ DMF): 402 nm; IR (KBr, ν_{max}, cm^{-1}): 3436 (N-Hstr), 3026 (Ar-H), 1708 (C=O str), 1493 (-N=N-), 1351 (C=S str), 1241 (C-O str), 1397 (NO_2 symstr), 1515 (NO_2 asymstr); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.15 (s, 1H, thiobarbituric acid NH), 11.55 (s, 1H, thiobarbituric acid NH), 14.25 (s, 1H, thiobarbituric acid OH), 7.48-8.12 (m, 4H, Ar-H), 2.17 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.886, 100; *m/z*; 291.89 (M-2); Analysis calcd. (%) for $C_{10}H_7N_5O_4S$: C, 40.96; H, 2.41; N, 23.88; S, 10.93. Found (%): C, 40.94; H, 2.39; N, 23.86; S, 10.95.

5-[(4-Carboxyphenyl)diazenyl]-2-thiobarbituric acid (4x): Yellow colour powder; Yield 75 %; R_f: 0.6, m.p. (°C): 295-305; UV-visible ($\lambda_{max,}$ DMF): 412 nm; IR (KBr, ν_{max}, cm^{-1}): 3428 (N-H str), 3235 (O-H str), 3085 (Ar-H), 1706, 1668 (C=O str), 1507 (-N=N-), 1161 (C=S str), 1241 (C-O str); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 12.95 (s, 1H, carboxylic acid OH), 11.35 (s, 1H, thiobarbituric acid NH), 11.55 (s, 1H, thiobarbituric acid NH), 14.45 (s, 1H, thiobarbituric acid OH), 7.65-8.15 (m, 4H, Ar-H), 3.77 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.672, 90.33; *m/z*; 290.89, (M-1); Analysis calcd. (%) for $C_{11}H_8N_4O_4S$: C, 45.20; H, 2.76; N, 19.17; S, 10.97. Found (%): C, 45.18; H, 2.74; N, 19.15; S, 10.95.

4-[(4,6-Dioxo-2-thioxohexahydropyrimidin-5-yl)-diazenyl]-N-(5-methylisoxazol-3-yl)benzenesulfonamide (4xi): Yellow colour powder; Yield 65 %; R_f: 0.8, m.p. (°C): 290-300; UV-visible ($\lambda_{max,}$ DMF): 412 nm; IR (KBr, ν_{max}, cm^{-1}): 3454 (N-H str), 2995 (Ar-H), 1685 (C=O str), 1503 (-N=N-), 1350 (C=S str), 1250 (C-O str), 1320 (SO_2 asymstr), 1157 (SO_2 symstr), 939 (S-N str); ^{1}H NMR (DMSO-d_6, δ ppm, 300 MHz): 11.15 (s, 1H, thiobarbituric acid NH), 12.49 (s, 1H, thiobarbituric acid NH), 14.23 (s, 1H, thiobarbituric acid OH), 7.54-7.73 (m, 4H, Ar-H), 7.40 (s, 1H, isoxazolyl-H-4), 12.42 (s, 1H, SO_2NH-), 2.50 (s, 3H, C-CH_3), 3.41 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.855, 100; *m/z*; 406.89 (M-2); Analysis calcd. (%) for $C_{14}H_{12}N_6O_5S_2$: C, 41.17; H, 2.96; N, 20.58; S, 15.70. Found (%): C, 41.18; H, 2.94; N, 20.84; S, 15.68.

5-[(2-Methylphenyl)diazenyl]-2-thiobarbituric acid (4xii): Orange colour powder; Yield 80 %; R_f: 0.8, m.p. (°C): 300-310; UV-visible ($\lambda_{max,}$ DMF): 420 nm; IR (KBr, ν_{max}, cm^{-1}): 3460 (N-H str), 2785 (Ali-CH_3), 1706, 1635 (C=O str), 1524 (-N=N-), 1351 (C=S str), 1258 (C-O str), 777, 842 (CH bend); ^{1}H NMR(DMSO-d_6, δ ppm, 300 MHz): 11.38, (s, 1H, thiobarbituric acid NH), 11.75 (s, 1H, thiobarbituric acid NH), 13.95 (s, 1H, thiobarbituric acid OH), 7.79-8.32 (m, 4H, Ar-H), 2.38 (s, 3H, tolyl), 2.77 (s, 1H, CH-N=N-); LC-MS (retention time, % area); 1.936, 100; *m/z*; 260.84 (M-2); Analysis calcd. (%) for $C_{11}H_{10}N_4O_2S$: C, 50.37; H, 3.84; N, 21.36; S, 12.23. Found (%): C, 50.36; H, 3.81; N, 21.32; S, 12.21.

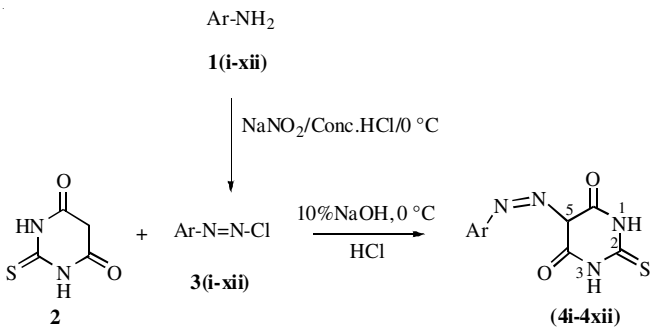
Antimicrobial activity: The above newly synthesized thiobarbituric analogues were investigated over different bacterial strains *viz*. *Escherichia coli* (MTCC 614), *Salmonella enterica ser.typhi* (MTCC 773), *Salmonella enterica typhimurium* (MTCC 98), *Salmonella enterica paratyphi* (MTCC 3220), *Shigella flexneri* (MTCC 1457), *Shigella flexneri* (MTCC 9543) *Pseudomonas aeruginosa* (MTCC 1035), *Vibrio cholera* (MTCC 3906), *Klebsiella pneumoniae* (MTCC 109), *Micrococcus luteus* (MTCC 1809), *Bacillus circulans* (MTCC 490), *Streptococcus mitis* (MTCC 2695) and *Pectobacteri*um *carotovorum* (MTCC 1428) were procured from the Institute of Microbial Technology and Gene bank (IMTECH), Chandigarh, India. Four microorganisms including three resistant strains *viz*., *Escherichia coli*$_{res.}$ (resistant to norfloxacin, ofloxacin, ampicillin, cefixime, nitrofurantoin), *Pseudomonas aeruginosa* strain f_1 (resistant to erythromycin, ampicillin and salbactam combination), *Staphylococcus aureus* $_{res}$ (resistant to norfloxacin, ofloxacin, ampicillin, cefotaxime) and *Bacillus subtilis* strain hswx88[17] were isolated in the Pharmaceutical Biotechnology Division of the University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Odisha, India were also incorporated. The antibacterial spectrum of the synthesized compounds was evaluated against some pathogens causing urinary tract infections and opportunistic infections in human. The microorganisms under test were freshly sub cultured on nutrient agar plates (Hi-Media) 24 h prior to the evaluation of antimicrobial activity. Standard drugs used for antimicrobial assay were ciprofloxacin (CF), gentamicin (G) and chloramphenicol (C) (Hi-Media) used as reference antibiotics (RA) against the Gram positive and Gram-negative bacterial strains. The antimicrobial evaluation was performed by agar well diffusion assay method[18]. 100 µL of freshly prepared inoculums was aseptically transferred to the surface of sterile agar plates and evenly spreaded. The wells (6 mm diameter) were made using sterile borer. For study of zone of inhibition the stock solution of above synthesized compounds and reference antibiotics were made using DMF. Each well was filled with a definite volume of test solution (25 µL, containing 25 µg). Then the plates were incubated for 24 h at 37 °C. The diameter of zone of inhibition was measured using the Hi-Antibiotic Zone Scale (Hi-Media) and those

having more than 8 mm were considered as moderately active. Each test compound was screened thrice against individual strains.

Stock solution of azo-2-thiobarbituric acid analogues and reference antibiotics were serially diluted by two-folds to obtain concentration ranges of 0.976-500 μg mL^{-1} and 0.61-78.2 μg mL^{-1} respectively, for determination of minimum inhibitory concentration (MIC)[19]. The minimum inhibitory concentration of the sample inhibiting the visible growth of a microbe was determined by the micro-broth dilution method according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

RESULTS AND DISCUSSION

Twelve new compounds of 5-(substituted phenyl diazenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**4i-4xii**) were synthesized by coupling of diazotized aniline derivatives/ heteroaryl amine 3i-3xii with 2-thiobarbituric acid in presence of nitrosylchloride (**Scheme-I**). A common character of Ar-N_2^+ is the highly electrophilicity of the nitrogen which undergoes simple azo-coupling reaction with appropriate nucleophiles.[20] The structure of 5-aryl azo thiobarbiturate analogues compounds were established on basis of their elemental analysis, IR, ^{1}H NMR, UV and XRD data. The majority of synthesized compounds were coloured and thermo stable. The compounds are soluble in basic polar solvents such as DMSO and dioxane and insoluble in organic non polar solvents.



Ar/heteroaryl; 4-sulfomoyl phenyl- (**4i**), 4-nitro phenyl- (**4ii**), 2-sulfonic phenyl- (**4iii**), 4-methoxy phenyl- (**4iv**), 4-bromo-3-methyl phenyl- (**4v**), 2-thiazolyl- (**4vi**), 2-methoxy phenyl-(**4vii**), 4-(1,5-dimethyl-2-phenyl-pyrazol-3-one- (**4viii**),3- nitro phenyl- (**4ix**), 2-carboxy phenyl- (**4x**), N-(5-methylisoxazol-3-yl)benzene sulfonamide- (**4xi**), 2-methyl phenyl- (**4xii**)

Scheme-I

In the IR spectra of compounds **4i-4xii**, the disappearance the absorption band at 2720 cm^{-1} due to CH_2 stretching of thiobarbituric acid, a new significant stretching vibration bands showed at 1507-1483 cm^{-1} due to formation and substituted diazo (–N=N-) in the C-5 position of 2-thiobarbituric acid. Also, the absence of the absorption band at about 2600-2500 cm^{-1} region indicates for SH group has proved that all the compounds were in the thionic form. All the products shows characteristic strong absorption band at 1355-1324 cm^{-1} due to C=S functional group in cyclic structure thioamide. The IR spectral data of the entire prepared product have been showed two strong absorption bands at 1706-1699 cm^{-1} and 1668-1640 cm^{-1} due to presence of the dicarbonyl in the structure. In addition to these bands, the compound **4i** showed three different vibrational bands at 1320, 1175 and 905 cm^{-1} indicate the presence of SO_2 str and S-N str in sulfonamide respec-tively[21]. The overlay in FT/IR spectra of compound **4iv** and **4v** is represented in Fig. 1.

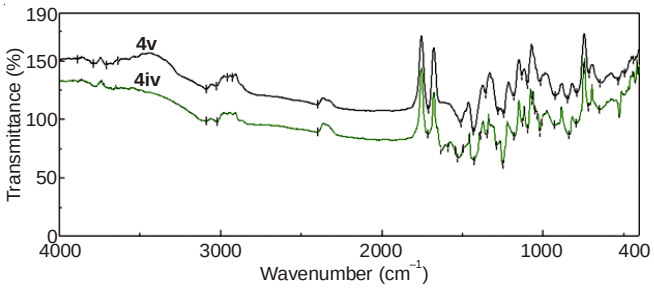


Fig. 1. Overlay in FT/IR spectral data of compound **4iv** and **4v**

In general, ^{1}H NMR spectra of unsubstituted thiobarbituric acid showed peak singlet at δ4.05 for two protons[22]. In all the prepared compounds, the amine (NH_2) functional group as well as methylene (CH_2 of 2-thiobarbituric acid) singlet peaks was not observed in ^{1}H NMR spectral data. ^{1}H NMR Spectra of the of synthesized compound 5-[(4-nitrophenyl) diazenyl]-2-thiobarbituric acid **4ii** showed two doublet at δ 7.79 and 8.32 ppm (J = 9.3Hz), two amide protons and highly deshielded NH/OH proton appeared at δ 11.42, 11.75 and 14.35 ppm respectively which all are comprised by ^{1}H NMR data of authentic compound[7]. The compound **4viii** showed two characteristic methyl peaks at δ 3.14 singlet and δ 2.17 singlet, which can attribute due to the presences of proton N-CH_3 and =C-CH_3 respectively. The ^{1}H NMR of compound **4xi** is represented in Fig. 2.

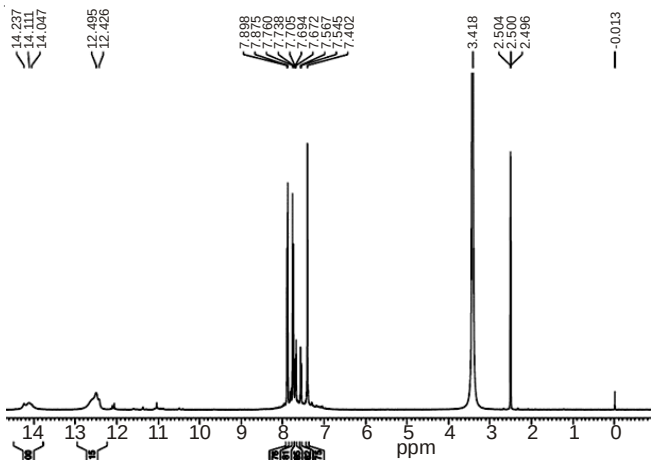


Fig. 2. ^{1}H NMR spectral data of compound **4xi**

The reported LC-MS spectrums of all synthesized compounds **4i-4xii** was revealed that molecular ion peaks which confirms strongly their predicted molecular formula. The compound **4 viii** at retention time 1.685 having base peak 359.13 strongly reveals the predicted molecular formula $C_{15}H_{14}N_6O_3S$ (Fig. 3).

The X-ray diffractogram of **4vi** and **4viii** consists of fourteen and eleven reflections between 10° to 50° and 10° to 70° respectively with maximum reflections at 2θ = 12.577° and 31.626° corresponding to value for d = 7.03 and 2.82 Å respectively. The X-ray diffraction pattern of compounds **4vi**

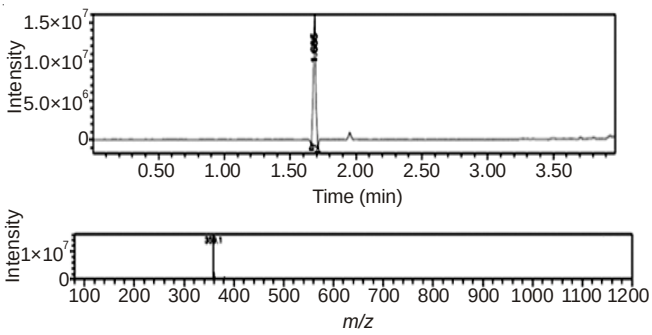


Fig. 3. LC-MS of compound **4viii**

and **4viii** with respect to their prominent peaks have been indexed by using commercially available software by trial and error method.

Thermal behaviour of the azo 2-thiobarbituric acid analogue DSC was performed. The peak temperature (255.67 °C) obtained from the DSC of the synthesized compound **4viii** corresponding to its melting point (250-260 °C). The sharp exothermic peak curve which represents the crystallization phase of the synthesized compound[23].

The UV-visible absorption spectra of compound **4i-4xii** were recorded over range of λ_{max} between 250-600 nm using a different of solvents at a concentration of 10^{-5} to 10^{-6} M (Table-1). This is done with intention of investigating the solvatochromic properties of these compounds. Due to π-π* electronic transition in azo groups, the maximum absorption lies in the range of 389 to 438 nm with the some variations. The electronic absorption spectra of the compounds in DMSO showed in each case the two absorption bands in the regions 450-415 and 280-250 nm. Such an absorption pattern is very similar to that reported for presence of substituted phenylazo chromophore[24]. According to UV data in Table-1, the compound **4viii**, insertion of 4-(-2-phenyl-1,5-dimethyl-3-oxo)pyrazolyl substituent into the thiobarbituric acid at the C-5 position gives the second largest bathochromic shift in acetone, DMSO and THF with respect to the λ_{max} compare to other polar solvents. In compound **4iv**, 4-anisyl azo substituent attached in 2-thiobarbituric acid ring showed the largest bathochromic shift in all six different solvents with respect to the λ_{max} compare to other synthetic dyes. The compound **4ii** showed also good bathochromic shift in DMF and DMSO solvent respect to the λ_{max}, this shift may due to presence of *p*-nitrophenyl azo substituent attached in same position. These bathochromic shifts can be attributed due to the interaction H-atom of amino proton of dyes with aprotic polar solvent such as THF and DMSO because of increase polarity of the dyes system, generally in the excited state. In other hand the presence of 2-thiazolyl substituent at the C-5 position in compound **4vi** gives rise to bathochromic absorption, when compare to sulfonamide substituted benzene derived azo **4i** in all six solvents[25]. The solvatochromism of all the newly synthesized 2-thiobarbituric acid congeners in DMSO and DMF are presented in Fig. 4.

The *in vitro* antimicrobial activity of the azo-thiobarbituric acid analogues was tested against different bacterial pathogens, some of which causes urinary tract infections. This was qualitatively and quan-titatively assayed by the mean diameter of inhibitory zones employing agar well diffusion assay as well

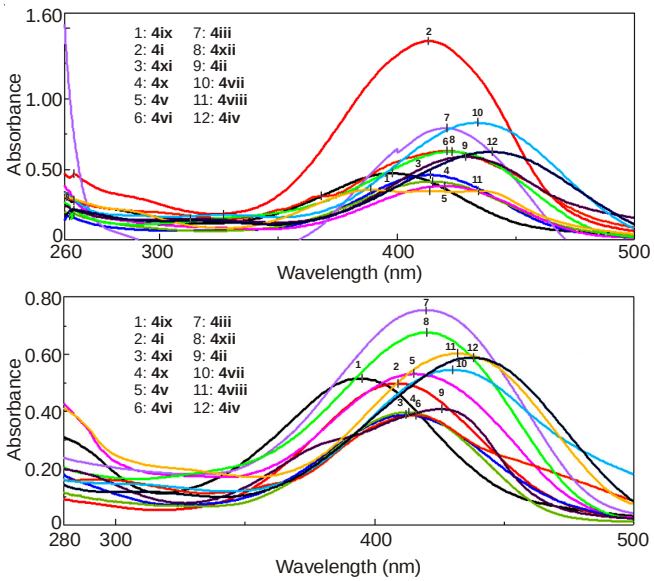


Fig. 4. Solvatochromic effect of thiobarbituric acid analogues using DMSO and DMF respectively

as determination of MIC using micro-broth dilution assay method. The anti-microbial potential of azo-2-thiobarbituric acid analogues were compared with reference antibiotics (gentamycin, ciprofloxacin and chloramphenicol), each taken as a positive control. The results of the antibiogram pattern and MIC values are summarized in Tables 2 and 3, respectively. The synthesized 2-thiobarbituric acid congeners **4i**, **4ii**, **4iii**, **4iv** and **4vi**, **4vii**, **4viii**, **4ix** are represented in Fig. 5, respectively against *S. mitis* and *K. pneumoniae*.

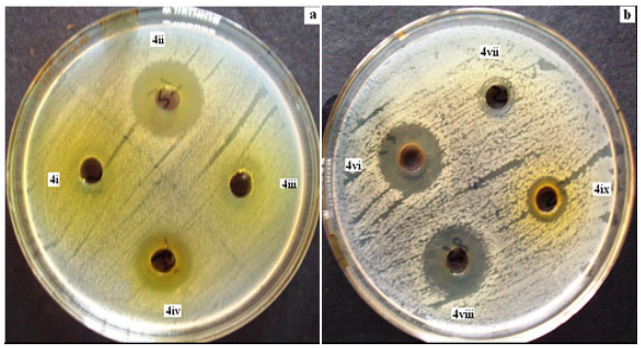


Fig. 5. Zone of inhibitions showed by compound (**4i, 4ii, 4iii, 4iv**) and (**4vi, 4vii, 4viii, 4ix**) against *S. mitis* (**a**) and *K. pneumoniae* (**b**) respectively

The results of antibacterial activities of the synthesized compounds (**4i-4xii**) against different bacterial pathogens revealed that the compounds **4ii, 4v, 4vi, 4vii, 4viii, 4ix** and **4xii** exhibited significant inhibitory activities against majority of pathogens when compared to the respective reference antibiotics. The inhibitory spectrum of the compounds may be attributed to the structural attachment of 4-nitro phenyl, 4-bromo, 3-methyl phenyl, 2-thiazolyl, 2-methoxy phenyl, 4-(1,3-dimethyl-2-phenyl-3-oxo pyrazolyl)-, 3-nitro phenyl and 2-methyl phenyl- substituent at C_5 position of azo 2-thiobarbituric acid nucleus respectively whereas the compounds **4i, 4iv, 4x** and **4xi** exhibited moderate significant inhibition against test pathogens in comparison to reference antibiotics. However,

TABLE-1
UV-VISIBLE SPECTRAL DATA (λ_{max}) OF SYNTHESIZED AZO-THIOBARBITURIC ACID ANALOGUES (**4i-4xii**) USING DIFFERENT SOLVENTS

Compound	Acetone	Ethanol	DMF	Dioxane	DMSO	THF
4i	406	-	265, 409	254, 328, 409,	264, 410	409
4ii	-	-	266, 430	-	429	-
4iii	-	416	419	415	421	-
4iv	234, 435	435	438	314, 437	264, 440	436
4v	414	-	265, 415	261, 419	264, 420	285, 416
4vi	360, 411	-	265, 416	272, 415	368, 421	414
4vii	429	-	265, 432	325, 430	327, 434	245, 427
4viii	435	422	265, 426	-	263, 435	-
4ix	397	-	265, 395	402	264, 398	400
4x	-	421	413	266, 412	264, 415	270, 411
4xi	405	413	266, 412	409	262, 414	296, 407
4xii	416	-	420	418	264, 423	417

TABLE-2
ZONE OF INHIBITION VALUES OF NEWLY SYNTHESIZED AZO-THIOBARBITURIC ACID ANALOGUES (**4i-4xii**) AGAINST VARIOUS BACTERIAL STRAINS EXPRESSED IN MEAN ± S.D.

Compound	Zone of inhibition (mm)								
	Ec	*Se*	*St*	*Sp*	*Sf_1*	*Sf_2*	*Pa*	*Vc*	*Kp*
4i	–	–	11.2±1.2**	11.0±0.5**	–	–	–	–	–
4ii	17.2±0.8	11.6±1.3**	12.5±0.5**	15.8±1.6	13.3±1.6**	24.2±1.6	10.5±1.5**	11.3±0.6*	15.9±0.6
4iii	–	–	–	–	–	–	–	–	–
4iv	16.2±1.2	–	–	11.4±0.6**	10.2±0.8**	21.1±1.4	–	–	15.6±1.6
4v	12.1±0.7**	11.6±0.6**	12.8±1.2**	11.6±1.6**	15.2±1.9	–	10.3±1.1**	–	13.6±1.8**
4vi	11.1±0.3**	–	8.2±0.8**	11.4±1.4**	11.0±1.2**	–	–	–	20.2±1.3**
4vii	15.1±0.8	9.2±1.3**	10.1±1.7**	12.5±1.0**	11.5±1.3**	19.3±0.6*	17.4±1.8	–	11.4±1.7*
4viii	14.6±0.5	11.8±0.2**	10.6±0.3**	12.8±1.4**	–	–	–	–	20.1±1.2**
4ix	12.8±1.1**	11.6±1.3**	10.2±0.2**	13.1±0.8**	–	11.3±1.6**	–	–	12.5±1.3**
4x	11.2±0.8**	–	–	10.1±0.2**	–	–	18.1±0.7	–	13.2±0.1**
4xi	–	–	10.2±0.2**	–	12.9±0.3**	–	–	–	13.7±0.5**
4xii	10.0±0.5**	11.4±1.6**	–	15.6±0.2	–	11.4±1.2**	–	–	20.2±0.3**
RA	16.1±0.6 (G)	23.0±0.3 (CF)	18.3±0.3 (CF)	16.7±0.5 (CF)	18.0±0.2 (CF)	22.5±0.1 (CF)	17.1±0.4 (G)	14.8±0.2 (C)	17.0±0.2 (G)
F value	35.776	53.547	32.199	13.629	15.243	63.942	31.433	9	30.186

Compound	Zone of inhibition (mm)							
	ML	*Bc*	*Sm*	*Pc*	*Bs*	*Pa(f_1)*	*Sa_{res}*	*Ec_{res}*
4i	8.2±1.4**	10.4±0.6**	11.2±1.4	10.1±0.7**	–	–	–	–
4ii	8.4±0.8**	22.3±1.3*	17.6±0.6**	14.8±1.2**	–	13.7±0.9*	16.7±1.4**	15.4±1.2
4iii	10.4±1.6**	–	12.2±0.6	–	–	11.4±1.6**	–	–
4iv	12.2±1.2	19.4±1.6**	13.2±0.8	13.5±1.5**	–	–	13.4±0.9**	14.2±1.6**
4v	17.2±1.6*	12.4±0.8**	11.8±1.4	26.6±1.0**	10.7±1.6**	8.1±0.4**	–	11.4±0.6**
4vi	10.5±0.5**	–	11.0±0.6	–	–	8.4±0.8**		10.9±0.9**
4vii	12.5±0.1	11.2±0.6**	14.6±0.4	–	14.7±1.2	10.5±0.7**	11.0±0.9**	13.7±1.8**
4viii	10.9±1.3*	14.2±1.2**	14.8±1.1	–	–	10.0±1.0**	12.1±0.9**	11.4±1.2**
4ix	14.2±1.3	18.2±1.2**	14.8±0.3	–	–	12.3±0.7**	–	13±0.3**
4x	–	–	9.4±0.2**	–	–	9.5±0.2**	–	–
4xi	–	–	9.2±0.4**	–	–	10.2±0.3**	10.4±0.6**	–
4xii	17.4±0.5**	17.6±0.8**	11.3±0.6	13.2±0.2**	10.3±0.4**	10.2±0.5**	12.8±0.9**	–
RA	14.1±0.1 (CF)	25.3±0.4 (CF)	13.4±0.4 (C)	23.2±0.1 (CF)	15.7±0.1 (CF)	15.7±0.4 (CF)	25.2±0.5 (C)	18.0±0.2 (C)
F value	24.688	76.399	8.504	144.33	21.746	25.201	95.33	13.817

– no zone of inhibition observed; **=P<0.05; **=P<0.01; ***=P<0.001;* for n=3; (Reference Standard Vs Test group). Ns = non-significant. Reference Standard was compared with Test groups (2-thiobarbituric acid analogues **4i-4xii**); *Ec- E. coli*, *Se- S. enterica ser.typhi, St- S. enterica typhimurium, Sp- S. enterica paratyphi*, *Sf_1- S. flexneri* (MTCC 1457), *Sf_2- S. flexneri* (MTCC 9543), *Pa- P. aeruginosa* (MTCC 1035), *Kp- K. pneumonia, ML- M. luteus*, *Vc- V. cholera, Bc- B. circulans*, *Sm- S. mitis,* Pc- *P. carotovorum,* Bs- *B. subtilis* strain hswx88, Pa(f_1)- *P. aeruginosa* strain *$F_{1\ res}$* (resistant to Erythromycin, Ampicillin + Salbactam combination), *Sa- S. aureus $_{res}$* (resisitant to norfloxacin, ofloxacin, ampicillin and cefotaxime) and *E. Coli $_{res}$* (resistant to norfloxacin, ofloxacin, ampicillin, cefixime and nitrofurantoin). C: chloramphenicol; G: gentamycin; CF: ciprofloxacin.

the compound **4iii** exhibited least inhibitory zones against the test pathogens may be due to presence of 4-sulfonic phenyl attached to azo 2-thiobarbituric acid nucleus. The compounds having zones < 10 mm considered least sensitive and those having inhibitory zones ≥ 10 mm were considered as sensitive and further subjected to MIC determination.

TABLE-3
MIC VALUES SHOWN BY NEWLY SYNTHESIZED AZO-THIOBARBITURIC ACID ANALOGUES (**4i-4xii**) AGAINST VARIOUS BACTERIAL STRAINS

Compd.	Microorganisms MIC values (μg mL^{-1})																
	Ec	*Se*	*St*	*Sp*	*Sf$_1$*	*Sf$_2$*	*Pa*	*Vc*	*Kp*	*ML*	*Bc*	*Sm*	*Pc*	*Bs*	*Pa(f_1)*	*Sa$_{res}$*	*Ec$_{res}$*
4i	–	–	250	500	–	–	–	–	–	ND	NF	500	NF	–	–	–	–
4ii	500	250	250	125	15.62	3.9	250	125	7.81	ND	7.81	15.62	62.5	–	125	62.5	125
4iii	–	–	–	–	–	–	–	–	–	NF	–	250	–	–	125	–	–
4iv	7.8	–	–	250	NF	3.9	–	–	15.62	250	31.25	125	31.25	–	–	250	125
4v	125	500	250	500	15.62	–	500	–	125	62.5	250	500	7.81	250	ND	–	125
4vi	500	–	NF	125	250	–	–	–	7.8	125	–	NF	–	–	ND		250
4vii	1.25	ND	NF	500	250	7.81	15.62	–	125	250	250	250	–	125	500	NF	62.5
4viii	250	500	NF	500	–	–	–	–	7.8	500	125	125	–	–	NF	250	250
4ix	250	500	NF	250	–	125	–	–	250	62.5	7.81	62.5	–	–	125	–	62.5
4x	250	–	–	NF	–	–	15.62	–	62.5	–	–	ND	–	–	ND	–	–
4xi	–	–	500	–	250	–	–	–	125	–	–	250	–	–	250	500	–
4xii	500	125	–	3.9	–	250	–	–	3.9	31.25	15.62	125	62.5	NF	500	250	–
RA	4.88 (G)	2.44 (CF)	4.88 (CF)	9.76 (CF)	9.76 (CF)	9.76 (CF)	9.76 (G)	19.52 (CF)	4.88 (G)	4.88 (CF)	9.76 (CF)	4.88 (C)	19.52 (CF)	9.76 (C)	4.88 (CF)	4.88 (C)	9.76 (C)

Nd- MIC values not determined for organisms exhibiting inhibitory zones of 8-9 mm; NF-MIC values not found in the dose range of 500-0.97 μg mL^{-1} of Azo-2-thiobarbituric acid compounds; *Ec- E. coli*, Se- *S. enterica sertyphi, St- S. enterica typhimurium, Sp- S. enterica paratyphi*, *Sf$_1$- S. flexneri* (MTCC 1457), *Sf$_2$- S. flexneri* (MTCC 9543), *Pa- P. aeruginosa* (MTCC 1035), *Kp- K. pneumonia, ML- M. luteus*, *Vc- V. cholera, Bc- B. circulans, Sm- S. mitis, Pc- P. carotovorum, Bs- B. subtilis* strain hswx88, *Pa(f_1)- P. aeruginosa* strain *$f_{1\ res}$* (resistant to Erythromycin, Ampicillin + Salbactam combination), *Sa- S. aureus $_{res}$* (resisitant to norfloxacin, ofloxacin, ampicillin and cefotaxime) and *E. Coli $_{res}$* (resistant to norfloxacin, ofloxacin, ampicillin, cefixime and nitrofurantoin); MIC- Minimum Inhibitory Concentration; C: chloramphenicol; G: gentamycin; CF: ciprofloxacin.

The synthesized azo-2-thiobarbituric acid analogues exhibited optimum inhibition against *K. pneumoniae* followed by *S. enterica paratyphi* and *S. mitis* and moderate inhibition against *E. coli, B. circulans* and *M. luteus.* However, *V. cholera* was found to be resistant. The compounds responded moderate antibacterial activity against the isolated resistant organisms *viz*. *E. coli$_{res.}$* followed by *S. aureus$_{res}$* and *B. subtilis* hswx88. The 2-thiobarbituric acid analogues also exhibited good antibacterial activity by inhibiting *S. enterica typhimurium* followed by *S. flexneri*, *S. flexneri*, *P. carotovorum* and *S. enterica ser.typhi.* The synthesized azo-2-thiobarbituric acid analogues exhibited broad spectrum antibacterial potential against test pathogens, some of which are causing urinary tract infections[18]. However, the compounds were also found to be sensitive against the three resistant strains. The compounds [(**4v** and **4vii**), (**4ii**, **4iv** and **4vii** and **4viii**) and (**4ii, 4iv, 4vii** and **4ix**)] inhibited *B. subtilis* strain hswx88$_{res.}$, *S. aureus$_{res.}$* and *E. coli$_{res.}$*, respectively to a greater extent. The antibiogram pattern of the synthesized compounds having inhibitory zones ≥ 10 mm, were confirmed by determination of MIC values employing micro-broth dilution method.

The inhibitory property of the azo-2-thiobarbituric acid analogues was determined in terms of MIC (μg mL^{-1}) within a concentration range of 0.976-500 μg mL^{-1}. The MIC values of the azo-2-thiobarbituric acid analogues against the test pathogens were shown in Table-3.

The azo-2-thiobarbituric acid analogues **4ii, 4iv** and **4xii** exhibited potential antibacterial activity by inhibiting the growth of *S. flexneri* (*f_1*), *S. flexneri* (*f_2*) and *S. paratyphi; K. pneumoniae* respectively at a concentration of 3.90 μg mL^{-1} as compared to the MIC values of 9.76; 9.76; 9.76 and 4.88 (μg mL^{-1}) registered by reference antibiotics. Interestingly, the azo-2-thiobarbituric acid analogues with comparatively lower MIC values, claimed to be potential anti-bacterial than reference antibiotics, against the above specified pathogens.

The azo-2-thiobarbituric acid analogues *viz*., (**4ii, 4iv, 4v, 4vi, 4vii, 4viii** and **4ix**) inhibited *K. pneumonia, E. coli*, *P. carotovarum, K. pneumoniae, S. flexneri* (*f_2*), *K. pneumoniae* and *B. circulans* at concentration of 7-81 μg mL^{-1}, when compared to reference antibiotics respectively. The compounds **4v** and **4ix** inhibited the growth of *P. carotovorum* and *B. circulans* at a comparative lower concentration of 7.81 when compared to reference antibiotics, having MIC values of 19.52 and 9.76 μg mL^{-1} respectively. The compound **4ii** inhibited *S. aureus$_{res.}$* and *P. aeruginosa* strain *$f_{1\ res}$* at MIC value of 62.5 and 125 μg mL^{-1}, respectively. The compound **4vii** inhibited the resistant strain *E. coli$_{res.}$* at MIC values of 62.5 μg mL^{-1} and the compound **4ix** inhibited *E. coli$_{res.}$* at MIC value of 62.5 μg mL^{-1}. The compounds **4iii** and **4ix** also inhibited *P. aeruginosa* strain *$f_{1\ res}$* at MIC values of 125 μg mL^{-1}. Finally it was concluded that the research work includes interesting technique and easy execution of simple one step synthesis, to provide twelve bioactive molecules against various pathogens and their successful spectral characterizations. These compounds were explored to their *in vitro* antimicrobial activity and further they can be used as ligands for new azo metal complexes. The effect of electron withdrawing groups such as nitro, bromo, special group like N-substituted sulfonamide of phenyl and some active heteroaryl pharmacophores in 2-thiobarbituric acid ring system as unique structural features might be responsible for antibacterial potential.

Conclusion

The work included with twelve newly synthesized azo thiobaituric acid analogues showing potential antibacterial activity against a wide range of bacterial strains out of which few are causing UTIs. The structure and composition of all the ligands are confirmed by different modern analytical techniques. The solvatochromic effect of the novel 2-thiobar-

bituric acid analogue **4viii** revealed that the insertion of 4-(-2-phenyl-1,5-dimethyl-3-oxo)pyrazolyl substituent into the thiobarbituric acid at the C-5 position showed the second largest bathochromic shift in acetone and DMSO with respect to the λ_{max} compare to other polar solvents used.

ACKNOWLEDGEMENTS

The authors acknowledge to Dean, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Director of NISER, IMMT and Deputy Director RIAES, Bhubaneswar, India.

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