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Synthesis, Characterization, and Antimicrobial Evaluation of New Heterocyclic Compounds Bearing a Biologically Active Sulfadiazine Moiety

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ABSTRACT

The eco-friendly techniques were utilized for the straightforward synthesis of new heterocyclic compounds bearing the sulfadiazine moiety by employing ultrasound irradiation techniques. Initially, sulfadiazine (1) was subjected to diazotization, followed by a coupling reaction with an activated methylene group (ethyl acetoacetate) to yield a hydrazone compound (3). Subsequently, the coupling adducts (3) were reacted with ethyl benzoylacetate, benzyl hydrazine, thiosemicarbazide, and guanidine under ultrasound irradiation, leading to the formation of pyridazine (4), pyrazolines (5, 6), and pyrimidine (7), respectively. Furthermore, when pyrazoline compound (6) was treated with chloroacetone and bromoaceophenone under ultrasound conditions, thiazole compounds (6a) and (6b) were formed, respectively. Additionally, new derivatives (10) and (11) were synthesized through diazotizing compound (7), followed by coupling reactions with an activated methylene group (acetylacetone) and reactions with urea and thiourea, respectively, all under ultrasound conditions. All the synthesized compounds were characterized using spectroscopic techniques such as FT IR, ^{1}H-NMR, and ^{13}C-NMR spectra. The synthesized chemicals exhibited promising antimicrobial activities against human pathogenic bacteria strains (Escherichia coli and Staphylococcus aureus) and Candida albicans fungi, as assessed through the broth microdilution technique.

1. Introduction

Sulfadiazine (SDZ) is one of the most important antibiotics in medicines, which is known as a sulfa drug or the sulfonamide group. According to the IUPAC system, this compound is called 4-amino-N-pyrimidin-2-yl-benzenesulfonamide. The chemistry of sulfonamide has a role in its manufacture in various forms as well as its integration with other compounds to increase its effectiveness against different diseases (Oudah et al., 2020).

Sulfadiazine is another frequently employed sulfonamide drug that is used in combination with the anti-malarial drug pyrimethamine to treat toxoplasmosis in warm-blooded animals (Ovung and Bhattacharyya, 2021).

Sulfonamide derivatives have been the focal point of consideration for scientists and researchers for quite a while because of their wide group of biological activity. A new candidate was generated from a combination of a sulfonamide group with other known scaffolds. This leads to an extension of the use of the sulfa drug to cover disease rather than bacterial infections such as Alzheimer's disease (Bowers et al., 2009). The metal complexes of sulfonamides exhibit an antibacterial activity higher than that of free parent sulfonamide (Topala et al., 2019). Heterocyclic sulfonamide compounds are utilized as carbonic anhydrase inhibitors (Smaine et al., 2008), diuretics (Supuran and Scozzafava, 2000), anti-human immunodeficiency virus (HIV) (Elsaid, 2011).

Furthermore, A wide variety of structurally unique sulfonamides were recently found to exhibit significant in-vitro and in-vivo anticancer efficacy (Alqasoumi et al., 2010) inhibition (Huang et al., 2006). The sulfonamide group, which is connected to the acetamide moiety and contains diverse aryl and heteroaryl substituents, has strong pharmacological activity, and sulfonamide derivatives containing brief amine fragments show promising anticancer action (Al-Said et al., 2011), antibacterial (Argyropoulou et al., 2009, El Sehrawi et al., 2017), antimicrobial (Naaz et al., 2018), as inhibitor (Kılıcaslan et al., 2016). Thiazole combined with other chemical compounds produces significant biologically potent derivatives that have been reported to exhibit a wide spectrum of biological activities such as antimicrobial, anti-inflammatory, antioxidant, analgesic, anticancer, antiviral, anti-HIV, antidiabetic, anticonvulsant, antimalarial and antitubercular (Mech et al., 2021, Osmaniye et al., 2018, Tratrat et al., 2022). On the other hand, Sulfadiazine is considered as one of the most important antibiotics that is used in treatment of many diseases such as urinary tract infections (UTIs), toxoplasmosis, malaria (Fahad et al., 2021). Sulfadiazine compounds with guanidinyl functionality showed excellent antituberculosis activity, the resonance stabilization of guanidine group was mainly responsible for the observed activity and different groups on N-substituted phenyl rings of guanidinyl influence the activity (BHAT et al., 2018). The nitrogen containing heterocycle pyridazine is a key intermediate in the synthesis of several fused heterocycles used in drug discovery (Bel Abed et al., 2013). Many pyrazole derivatives are acknowledged to possess a wide range of bioactivities, thus, some representatives of this heterocycle exhibit anti-viral/anti-tumor (Karrouchi et al., 2018), antibacterial (Mehta et al., 2017), anti-inflammatory (Penning et al., 1997).

In this investigation, we have synthesized a series of new heterocyclic compounds bearing sulfadiazine moiety. Among the synthesized compounds, pyridazine, pyrazoline, thiazole, pyrimidine and thiazine derivatives through multi step reaction under ultrasonic conditions at 60 °C, which is a technique that uses high-frequency sound waves to accelerate chemical processes in a rapid and ecologically benign way (Fiego et al., 2021, Gharat and Rathod, 2020). All the synthesized compounds were characterized by spectroscopic techniques such as FT IR, ^{1}H-NMR, and ^{13}C-NMR spectra. *In vitro,* antimicrobial activity was determined against antibacterial, Gram-positive (*Streptococcus pneumoniae*), Gram-negative (*Escherichia coli*) and antifungal activity versus fungal strains (*C. albicans*).

2. Experimental

2.1 The chemicals and materials

All the reagents and reactants were obtained

from commercial sources and used without further purification unless otherwise mentioned. Sigma Aldrich supplied most of the chemicals (India). The sulfadiazine moiety underwent Ultrasonic-assisted reactions using electronics ultrasonic cleaner JP-060S. An uncorrected Stuart SMP10 digital melting point instrument was used to determine melting points. With a Shimadzu FT-IR 8400S infrared spectrophotometer, IR spectra were captured on KBr disks. 1H NMR spectra was obtained using a Bruker AVANCE III Nano Bay 400 MHz FT-NMR spectrophotometer at ^{1}H NMR (101 MHz, CDCl3). ^{13}C - NMR spectra were acquired using a Bruker AVANCE III Nano Bay 400 MHz FT-NMR spectrophotometer at ^{13}C NMR (101 MHz, CDCl3).

2.2 Synthesis of Ethyl3-oxo-2-(2-(4-(N-(pyrimidin2yl)sulfamoyl)phenyl)hydrazine ylidene)butanoate (3)

Step.1 Diazonium salt solution preparation (2)

A combination of sulfadiazine (1)(0.01mole) in concentrated HCl (3 mL) was chilled to 0 - 5°C in ice bath, and a dropwise addition of cooled sodium nitrite solution (1.5 g in 10 mL of water) was made for 10 minutes. Following that, the outcome of the mixture was agitated for 30 minutes (Saleh et al., 2003).

Step .2 Coupling reaction

A solution of diazonium salt compound (2) (0.01mole) was added dropwise while stirring into an ice-cold combination of the relevant methylene compound ethyl acetoacetate (0.01mole) and sodium acetate (4.10 g; 0.05 mole) in ethanol (50 mL). After 30 minutes continuous stirring, the reaction mixture had been kept at room temperature for 2h to get the correct hydrazone derivative (3), the product has been recovered and recrystallized from absolute ethanol to give Sparkling yellow crystals : yield (84 %); Mp. 167-168℃; IR (KBr, cm^{-1}): 3462 (NH),2925 (C-H aliph. str), 1726 (C=O str), 1647 (C=N) and 1338 (SO_2, asym), 1116 (SO_2 sym,); δ ^{1}H NMR (400 MHz, CDCl3). 2.60 (s, 3H, CH_3), 2.52 (t, 3H, CH_3), 4.35 (q, 2H, -CH_2-), 8.68 – 7.00 (m, 3H, pyrimidine +, 4H, Ar-H), 12.68 (s, H, NH). ^{13}C NMR (101 MHz, CDCl3) δ 14.20(CH_3), 27.04(CH_3), 62.00 (CH_2), 158.70-115.04 (4C-pyrimidine + 6C-Ar), 164.55(C=O), 194.37 (C=O).

2.3 General procedure for the synthesis of compounds (4-11)

2.3.1 Synthesis of compound (Ethyl5-benzoyl-4-methyl-6-oxo-1-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)-1,6-dihydropyridazine-3-carboxylate) (4)

Compound 3 (0.005mole) and ethyl benzoylacetate (0.005mole) in dioxane (40 ml) with triethylamine (5 drops). Before being concentrated and cooled, the reaction mixture was ultrasonically heated for 35 minutes at 60 °C. The isolated solid component was filtered and recrystallized from ethanol to produce yellow color crystals of compound (4) (Azab et al., 2013). Yield: (70%); Mp. 235-237℃; FT-IR (KBr, cm^{-1}): 3458(NH), 2814 (C-H aliph. str.), 1683,1662 (C=O, COO), 1533 (C=N) and 1328-1182 (SO2). ^{1}H NMR (400 MHz, CDCl3) (δ/ ppm) 1.42 (s,3H, CH_3) 2.52 (t,3H, $COOCH_2CH_3$),4.39 (m,2H,CH_2) 8.71 - 7.03 (3H, Pyrimidine + 4H, Ar-H), 12.71(s, H, NH). ^{13}C NMR (101 MHz, CDCl3) (δ/ ppm) 14.06(CH3),14.30 (CH_3),61.94 (CH_2), 145.42(N=C-), 156.75(-COO), ,164.40 (CO), 156.72- 115.85 (4C-pyrimidine + 6C-Ar), 197.58(CO-Ph).

2.3.2 Synthesis of pyrazoline compound (4-((1-benzoyl-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide)(5)

A mixture of hydrazone 3 (0.01mole) and benzyl hydrazine (0.01mole) in dioxane (20ml) was ultrasonically heated for 35 minutes at 60 °C, concentrated, and chilled. The water-washed filtered soled products were dried and recrystallized from ethanol to produce yellow color crystals compound (5) ; (El-Gaby et al., 2002). yield (80 %); MP. 233℃, FT-IR (KBr, cm $^{-1}$): 3390 (NH), 2949, 2872 (C-H aliph. str.),), 1691,1647 (C=O str.), 1581 (C=N) and 1384-1182 (SO_2). ^{1}H NMR (400 MHz, CDCl3) δ 1.44 (s, 3H, CH3), 8.71 –7.03 (m, 3H, pyrimidine + 9H, 2Ar-H), 11.15 (s,1H, NH), 13.18 (s,1H, NH). ^{13}C NMR (101 MHz, CDCl3) δ 11.87(CH_3), 156.68 - 115.21 (4C- pyrimidine+ 12C-2Ar), 129.04(N-C=), 145.04(=C-CH_3), 158.59,157.39

(2 CO).

2.4 Synthesis of pyrazoline compound (5-methyl-3-oxo-4-((4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)diazenyl)-2,3-dihydro-1H-pyrazole-1-carbothioamide) (6)

To compound 3 (0.01mole), add thiosemicarbazide (0.01mole) and triethylamine (0.5ml) in 40ml dioxane. The mixture was heated under ultrasonic conditions at 60 °C for 40 minutes, concentrated, and chilled. The isolated solid component was filtered, washed several times using water and recrystallized from ethanol to produce yellow color crystals of compound (6), (El-Gaby et al., 2002).Yield (88%); MP. 223℃; FT-IR (KBr, cm^{-1}): 3488, 3375 (NH, NH2), 1689 (C=O str), 2833 (C-H aliph. str.), 1575 (C=N) and 1354-1182(SO2).^{1}H NMR (400 MHz, CDCl3) (δ/ ppm) 1.36 (s,3H, CH_3), 4.38 (s, 2H, NH_2), 8.68-7.00 (m, 3H, Pyrimidine + 4H-Ar), 11.87 (s,1H, NH), 12.69 (s,1H,NH). ^{13}C NMR (101 MHz, CDCl3) (δ/ ppm) 13.97 (CH3), 136.69 (N-C=),144.37, 156.76-114.90 (4C-pyrimidine +6C -Ar), 158.60 (CO), 163.41 (C=S).

2.4.1 Synthesis of compound 4-((5-methyl-1-(4-methylthiazol-2-yl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (6a)

A mixture of 6 (0.005 mole) and chloroacetone (0.01 mol) in 30 ml absolute ethanol was heated for 38 minutes under ultrasonic conditions at 60 °C.**).** The precipitate was filtered off, washed several times with cold water, dried, and recrystallized from absolute ethanol to give light-yellow crystals of compound (6a); (Gomha et al., 2015). Yield (79%); MP. 201℃; FT-IR (KBr,cm -1): 3454 (NH), 1691 (C=O str), 2926 (C-H aliph. str.), 1583 (C=N) and 1336-1184 (SO2).1H NMR (400 MHz, CDCl3) δ 1.44 (s,3H, CH3), 2.65 (s,3H, CH3), 8.67-7.05 (m, 3H, Pyrimidine + 4H-Ar), 11.33 (s,1H, NH),13.78 (s,1H, NH). 13C NMR (101 MHz, CDCl3) δ 24.69 (CH3), 27.04 (CH3), 136.69 (N-C=), 156.63-114.90 (4C-pyrimidine +6C -Ar), 156.62 (CO), 165.00 (S-C-N).

2.4.2 Synthesis of compound 4-((5-methyl-3-oxo-1-(4-phenylthiazol-2-yl)-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-N-(pyrimidin-2-yl) benzenesulfonamide (6b)

A solution of compound 7 (0.005 mol) and bromoaceophenone (0.01 mol) and 30 ml ethanol was heated under ultrasonic conditions at 60 °C for 40 minutes. The end result began to separate throughout the reaction. (Lv et al., 2011). The solid product was then filtered, washed with water and recrystallized using ethanol to obtain pure 6b as a yellow crystal; yield (89%); MP. 198℃; FT-IR (KBr, cm^{-1}): 3446 (NH), 1705 (C=O str), 2733 (C-H aliph. str.), 1620 (C=N) and 1325-1182(SO2).1H NMR (400 MHz, CDCl3) δ 1.36 (s,3H, CH3), 8.70-7.00 (m, 3H, Pyrimidine + 9H-2Ar), 11.48 (s,1H, NH),12.69 (s,1H, NH). 13C NMR (101 MHz, CDCl3) δ 13.97 (CH3), 114.58 (S-C=) 136.69 (N-C=), 156.63-115.75 (4C-pyrimidine +12C -Ar), 158.58(CO), 163.39 (S-C=N).

2.4.3 Reaction of compound (3) with Guanidine, formation of compound 4-((2-amino-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (7)

Compound 3 (0.005mole) and guanidine (0.01mole) were dissolved in 50 % ethanol before adding sodium ethoxide (0.23 g Na metal in 10 ml ethanol). The solution has been heated under ultrasonic conditions for 35 minutes at 60 °C, then cooled before being poured into 50ml of cold water and acidified with HCl. The precipitate was filtered off, washed several times with cold water, dried, and recrystallized from absolute ethanol to give yellow crystals of compound (7); (Moustafa et al., 2003). Yield (85%); MP.198-199℃; FT-IR (KBr, cm^{-1}): 3481,3242 (NH, NH2), 1726 (C=O str.), 2833 (C-H aliph. str.), 1575 (C=N) and 1354-1182(SO_2).^{1}H NMR (400 MHz, CDCl3) δ 1.36 (s,3H, CH_3), 4.37 (s, 2H, NH_2), 8.68-7.01 (m, 3H, Pyrimidine + 4H-Ar), 11.87, 12.70 (s,1H, 2NH). ^{13}C NMR (101 MHz, CDCl3) δ 13.84(CH_3), 136.69 (N-C=), (=C-S), 156.63-114.90 (4C-pyrimidine +6C -Ar), 158.63 (-C-NH_2), 163.41(CO).

2.4.4 Diazotization of compound (7) for the synthesis of compound (8)

A compound (7) (0.01 mole) was dissolved in a (3 mL) of 3M hydrochloric acid and the solution

was cooled in an ice bath to 0-5 Cº. added slowly prepared solution of NaNO2 (2.17M, 10 mL), with stirring in which the temperature remains below 5C º. The solution was kept in the ice bath and immediately preceded to the next step to produce Diazonium (8). (Saleh et al., 2003).

2.4.5 Synthesis of compound 4-((2-((2,4-dioxopentan-3-yl)diazenyl)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide)(9)

Diazonium salt of compound (8) (0.01mol) was added dropwise in an ice-cold solution of acetylacetone (0.01mole) and sodium acetate (4.10 g; 0.05 mole) in ethanol (50 mL). After 30 minutes of stirring, the mixture has been kept at room temperature for 2 hours to get pale yellow of compound (9); (Saleh et al., 2003). Yield 77 %, MP. 209℃. FT-IR (KBr, cm^{-1}):3435 (NH),2880 (C-H aliph. str.), 1731 (C=O str), 1633 (C=N), and 1355-1139 (SO_2). ^{1}H NMR (400 MHz, CDCl3) δ 2.53 (s,H, CH_3), 2.64 (s,6H, $2CH_3$), 8.62-7.01 (3H, pyrimidine, 4H-Ar-H), 10.24 (s,1H, NH), 12.56 (s, 1H, NH). ^{13}C NMR (101 MHz, CDCl3) δ 26.70 (CH3),31.87 (CH_3), 130.55(C-CO-CH_3), 155.75 – 115.99 (4C-pyrimidine, 6C-Ar), 156.50 (N-C=N), 158.54 (CO).

2.4.6 Synthesis of compound 4-((2-((4,6-dimethyl-2-oxo-1,2-dihydropyrimidin-5-yl)diazenyl)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide)(10)

Add urea (0.01mole) and 40ml ethanol to compound 9 (0.005mole). Heat the solution under ultrasonic conditions at 60 °C for 25 m inutes, concentrated, and cooled before adding crushed ice and agitating for 1 hour. The solid component that had been isolated then filtered and recrystallized using ethanol resulted in the pure yellow color compound (10); (Saleh et al., 2003). Yield (86 %); MP. 223 ℃, FT-IR (KBr, cm^{-1}): 3462 (NH), 2884 (C-H aliph. str.), 1726 (C=O str), 1581 (C=N) and 1338-1161(SO2). ^{1}H NMR (400 MHz, CDCl3) δ 2.47 (s,3H,CH3),2.52 (s,3H, CH_3),2.62 (s,3H,CH3), 8.69 -7.04 (3H, pyrimidine + 4H, Ar -H), 11.79 (s, 1H, NH),12.71(s1H,NH), 14.57 (s, 1H, NH).^{13}C NMR (101 MHz, CDCl3) δ 14.06 (CH_3), 26.95 (CH_3), 30.94 (s,3H,CH3),156.72-114.90(4C,pyrimidine+ 6C,Ar), 145.58(N-C=), 158.63 (N-C=N), 163.32 (C=O), 164.41(C=O).

2.4.7 Synthesis of compound4-((2-((2-amino-4,6-dimethyl-4H-1,3-thiazin-5-yl)diazenyl)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl) diazenyl)-N-(pyrimidin-2-yl) benzenesulfonamide) (11)

In 50 absolute ethanol, compound 9 (0.005mole) and thiourea (0.01mole) were dissolved, then sodium ethoxide (0.23 g Na metal in 10 ml ethanol) was added. Heat solution for 30 minutes under ultrasonic conditions at 60 °C, and cooled before adding to 50ml of cold water and using HCl to acidify it. The precipitate was filtered off, washed several times with cold water, dried, and recrystallized from absolute ethanol to give Pall yellow crystals of compound (11); (Moustafa et al., 2003). Yield (82%); MP. 245 ℃, FT-IR (KBr, cm^{-1}): (3490-3034 cm^{-1}) (NH, NH2), 2879-2771 (C-H aliph. str.), 1670 (C=O str), 1567 (C=N) and 1378-1135(SO_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s,3H,CH3),2.36 (s,3H, CH3),2.78 (s,3H,CH_3), 4.43 (s,2H,NH_2), 8.48 - 7.02 (3H, pyrimidine + 4H, Ar -H), 9.36 (s, 1H, NH),11.12 (s1H,NH),.^{13}C NMR (101 MHz, $CDCl_3$) δ 16.61 (CH_3), 18.19 (CH_3), 20.10 (s,3H,CH3),156.72 -112.89 (4C,pyrimidine + 6C,Ar), 159.47 (S-C-NH_2),161.49 (NH-C=N),164.99 (CO).

2.5 Antimicrobial Assay

Synthesized compounds 3, 4, 5, 6, 6a, 6b, 7, 9, 10, and 11 were evaluated in vitro for antibacterial activity to the Gram-positive bacteria and Gram-negative bacteria, Staphylococcus aureus RCMB 0100010 (SA) and Escherichia coli CMB 010052 (EC), respectively. For antifungal activity, one fungal strain, Candida albicans RCMB 05036 (CA), is utilized. The inhibitory properties of the antibacterial and antifungal samples were evaluated using inhibition% ± standard deviation at a concentration of 100 µg/ml of tested samples. (Caldeira et al., 2013). After 24 hours, the optical densities of antimicrobials were measured at 37˚C in nutrient agar. Then the culture was added to the nutrient broth and left at 4°C. Overnight growing cultures

were extracted from the broth, and the turbidity of the culture was adjusted to 0.5 McFarland standards. At 105-107, CFU/mL and an optical density of 0.1 at 600 nm, 0.2 mL of cultivated microorganisms was increased to 20 mL of sterile nutritional broth. (Esma Gündüz et al., 2009)

2.5.1 Agar well diffusion assay

Mueller Hinton agar plates were continuously cultured using a cotton swab inoculated with bacterial and fungal strains. The petri dishes were left alone to absorb extra moisture. A sterile cork borer with an eight-mm diameter was utilized to produce 4 mm deep pits in the sealed agar medium. 150 µg/µL of each synthesized chemical substance was taken using a micropipette at varied concentrations (1000, 600, and 200 µg/µL) and applied to the pits on the plates. Positive controls Ciprofloxacin (5 µg/µL) and sterile distilled water were used as negative controls in the pits. After that the plates were incubated for 24 hours at 37˚C. The inhibitory zones in each sample, containing the pits, had been measured in millimeters using a caliper and the results were recorded. All experiments were carried out twice.

2.5.2 Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined using the broth microdilution method. To identify the lowest concentration of antimicrobial activity, the (18-24) hours isolated culture was standardized by comparing its turbidity with the 0.5 McFarland standard. The MIC against the studied microbial strains was determined using a 96 polystyrene well microtiter plate. Thereafter, 100 L of various concentrations of manufactured chemical compounds (1000, 600, and 200) µg/µL were added onto a series of microtiter plate wells. For comparison, 50 µl of the standardized inoculum suspensions were added into each test well, whereas the negative control well contained only broth and the positive control well included 50 µl microorganisms as well as the broth.

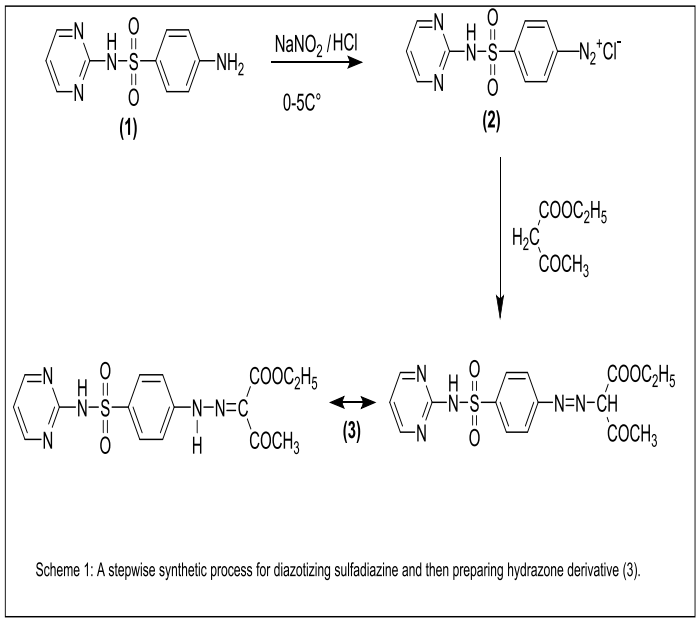
The microtiter plate pit was vortexed and incubated for 24 hours at 37°C. Eventually, transparent pits were chosen as the least generated chemical compounds concentration that suppressed bacterial growth when compared to control wells (Hamasalih and Abdulrahman, 2020, Wiegand et al., 2008)

3. Results and discussion

Diazotization is a process by which an aromatic primary amine is converted to a diazonium compound. In diazotization, sodium nitrite is added to a solution of the Sulphadiazine (1) in aqueous hydrochloric acid at 0–5°C, diazonium salts (2)was obtained which coupling reaction between active methylene (ethyl acetoacetate) and diazonium salts(2) is generally conducted at room temperature and in protic organic solvent in presence of base to afford compound (3). The explanation of this reaction is a nucleophilic attack of the active methylene of ethyl acetoacetate on the diazonium salt. (Scheme 1). FT- IR, ^{1}H-NMR, and ^{13}C-NMR data was used to describe compound (3). The IR spectra of (3) shows an absorption band at 3390 cm^{-1}, corresponding to the vibration of the NH group's, a band at 1726 cm^{-1} corresponds to the carboxylic ester, whereas bands at 1647 cm^{-1} belong to the acetyl for C=O group and band stretching band corresponding to carbon-carbon double bond of aromatic ring at (1411 and 1581 cm^{-1}). ^{1}HNMR spectrum of compound (3) in $CDCl_3$ showed the absence of the NH_2 group, while showed signals at 12.68 for NH group, 8.68 – 7.00 (m, 3H, pyrimidine +, 4H, Ar-H), 4.35 (q, 2H, -CH_2-), 2.60 (s,3H,$COCH_3$) and 2.52 (t,3H,CH_3 for carboxylic ester).^{13}CNMR (101 MHz, $CDCl_3$) showed δ 14.16(CH_3), 27.04(CH_3), 62.03(-CH_2), 158.75-115.00 (6C-Ar + 4C pyrimidine), 164.50 (C=O), 194.35 (COO).

This investigation resulted in synthesis of polyfunctional substituted pyridazine derivatives (4), was synthesized by cyclization of compound (3) with ethyl benzoylacetate which act as a nucleophilic center. The production of pyridazine derivative (4) ethyl5-benzoyl-4-methyl-6-oxo-1-(4-(N-(pyrimidin-2-yl)sulfamoyl)phenyl)-1,6-dihydropyridazine-3 carboxylate in an ultrasonic bath at 60 °C. It should be noted that the sonication assisted synthesis of test compounds resulted higher yields and shorter reaction times than conventional synthesis techniques.

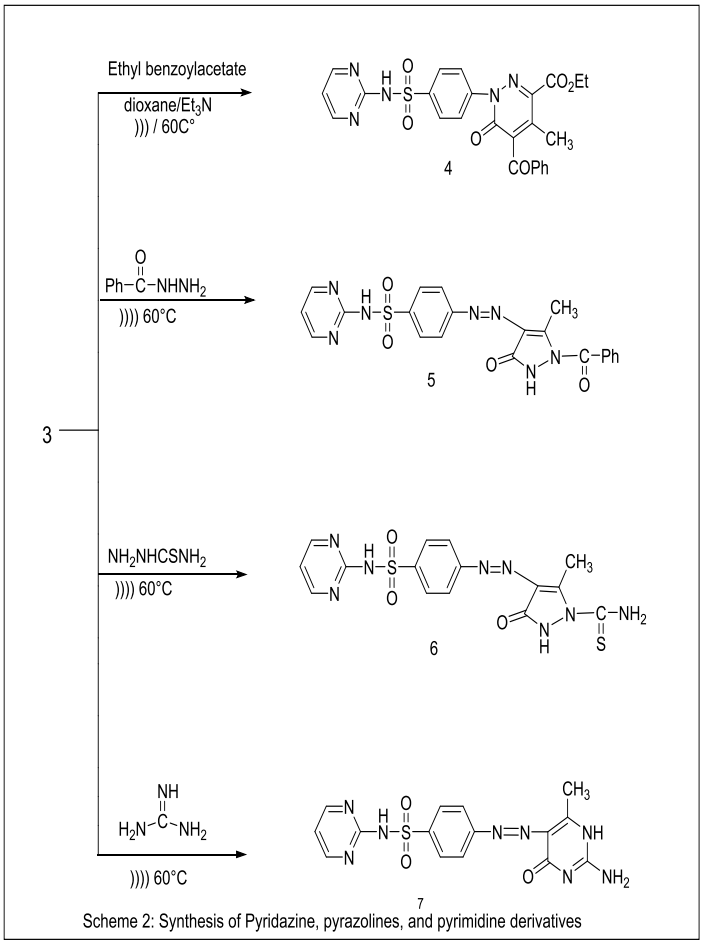
Compound (4) structure was determined using IR, ^{1}H-NMR, and ^{13}C-NMR. The structures of the produced molecule were determined using spectral data. IR spectra, distinguished by, attributed to exhibited a distinctive band for NH at 3458 cm^{-1},1683, 1662 cm^{-1} for CO, COO, and 2814, 2740 cm^{-1} for CH-aliphatic str. The ^{1}HNMR (400 MHz, $CDCl_3$) data for the synthesized compound showed a singlet at 12.70 for NH, 8.70-7.00 for (3H, Pyrimidine + 4H, Ar-H). Additionally, the proton signals of two groups of CH_3 appeared at 1.42 (s, 3H, CH_3) and 2.52 (t, 3H, $COCH_2CH_3$), revealing the absence of corresponding $COCH_3$ group bands. The ^{13}C NMR spectra for chemical (4) were reported, showing signals at 194.26 (-CO), 164.40 (COO), and 156.72-115.85 (6C-Ar + 4C-pyrimidine). Furthermore, signals at 14.06 and 14.30 were



Scheme 1: A stepwise synthetic process for diazotizing sulfadiazine and then preparing hydrazone derivative (3).

observed for ($2CH_3$). The^{1}HNMR and ^{13}CNMR data were assigned based on the comparison of the data obtained for compound (4) with those reported in the literature for pyridazine (Azab et al., 2013). ring system (Scheme 2)

Compound (3) were reacted with benzylhydrazine, cyclization nucleophilic addition occurs to give amino pyrazoles compound (5) using an ultrasonic bath at 60 °C (Scheme 2). Structure of the new compounds (5) was verified using IR, ^{1}H-NMR, and ^{13}C-NMR spectra (see experimental). The IR spectra of compound (5) revealed the vanishing of the characteristic band of the acetyl carbonyl groups and carboxylic acid ester, as well as the appearance of a strong band in 3390 cm^{-1}, attributed to NH group stretching, and the band of the pyrazolinonering C=O group appearing at 1691 $cm^{-1}$and 1647cm^{-1}. The ^{13}C NMR (101 MHz, $CDCl_3$) data for derivative (5) the carbonyl carbone of pyrazolinone and CO-Ph group appeard at 158.59 and 157.39 ppm and the signal for carbone CH_3 group appered at 11.88 ppm. The other carbon atoms in the pyrimidine, pyrazolinone, and phenyl all showed up at the predicted chemical changes (see Experimental).



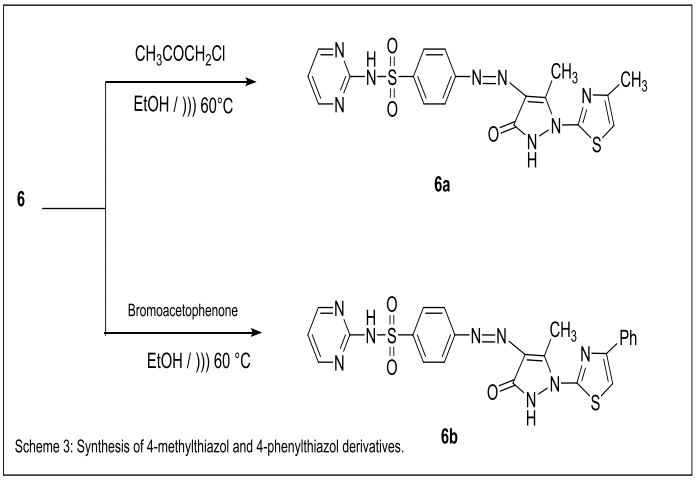
Scheme 2: Synthesis of Pyridazine, pyrazolines, and pyrimidine derivatives

The reaction of compound (3) with thiosemicarbazide (possessing nucleophilic properties) afforded the thiosemicarbazone which upon treating with and triethylamine in dioxane under ultrasonic conditions at 60 °C for 40 minutes cyclization nucleophilic addition accrue give a 5-methyl-3-oxo-4-((4-(N-(pyrimidine-2-yl)sulfamoyl)phenyl)diazenyl)-2,3-

dihydro-1H-pyrazole-1-carbothioamide (6). (Scheme 2).

The IR spectra of 6 were distinguished by the elimination of the acetyl carbonyl groups and carboxylic acid ester and the appearance of a strong band in 3488-3375 cm^{-1}, attributed to NH and NH_2 stretching groups. The infrared absorption caused by the C=S stretching vibration turned out to be attributed by a number of researchers to frequencies ranging from less than 800 cm-^{1} to higher than 1500 cm^{-1}. The important signal in the ^{1}H-NMR spectrum of 6) was singlet signals at 11.87 and 12.69 assigned to the two NH groups and a singlet at 4.38 attributable to the NH_2 protons. The ^{13}C NMR (101 MHz, $CDCl_3$) of 6 the appearance of C=O at 158.60 ppm. (Scheme 2). The synthesis of thiazolyl-pyrazoline derivatives (6a) and (6b) followed the general pathway outlined in (Scheme 3). The thiazol compounds 6a and 6b were obtained by heterocyclization compound (6) with substituted chloroacetone and 2-bromoaceophenone respectively to form 4-((5-methyl-1-(4-methylthiazol-2-yl)-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (6a) and 4-((5-methyl-3-oxo-1-(4-phenylthiazol-2-yl)-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfonamide (6b) under ultrasound conditions at 60 °C for 30-40 min. IR, ^{1}H-NMR, and ^{13}C-NMR were used to determine the structure of chemicals 6a and 6b. The IR spectra were distinguished by apery only one peck at 3487 cm^{-1} due to the stretching vibration of the NH group and elimination of the NH_2, C=O at 1660 cm^{-1}, and C-S at 600 cm^{-1} (see Experimental). The important signals in the ^{1}H-NMR spectrums of 6a and 6b were singlet signals at 11.33 and 13.78, which were assigned to the two NH group protons. The ^{13}C NMR (101 MHz, $CDCl_3$) of 6a revealed the absence of S=C-NH_2 groups as well as the emergence of the pyrazolinone methyl groups at 24.69 and 27.04 ppm. The other carbon atoms in the pyrimidine, pyrazolinone, and phenyl moieties all showed up at the predicted chemical changes (see Experimental). (Scheme 3)

Heterocyclization neophiliac addition of hydrazone compound (3) with guanidine (act as nucleophilic) for 35 minutes under ultrasonic conditions at 60 °C in ethanolic sodium ethoxide to produce pyrimidine derivatives (7) (Scheme 2). Compound (7)'s IR spectra indicated a typical band for NH_2 and NH at (3481, 3324) cm^{-1} and C=O at 1726 cm^{-1}. The lack of $COCH_3$ and $COOC_2H_5$ was shown by the ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 7, as well as the presence of wide singlet at 4.40 ppm for NH_2, 11.87 and 12.70 for 2 NH groups, 8.65 -7.01 (4H, Ar-H, + 3H- Pyrimidine), and 1.36 for (CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) analysis shown spectra,



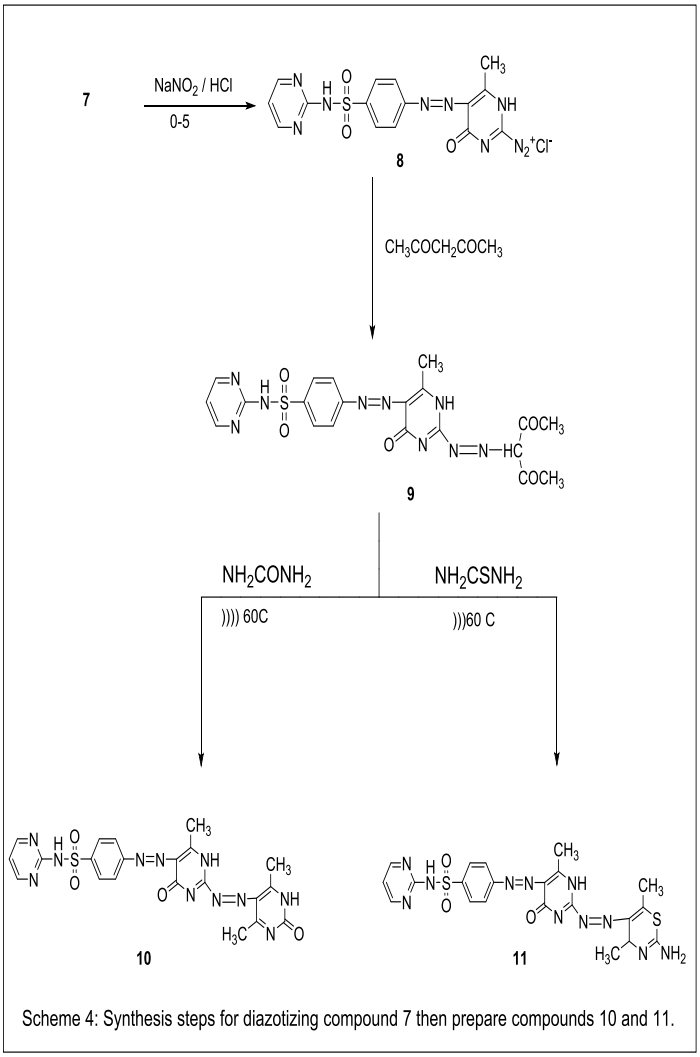
Scheme 3: Synthesis of 4-methylthiazol and 4-phenylthiazol derivatives.

165.01 (C=O), 156.63-114.90 (6C-Ar, 4C-pyrimidine), and 13.87 (CH_3).

At 0-5°C, the chemical (7) diazotizes with a sodium nitrite/hydrochloric acid mixture. To make the required hydrazone derivative (8), diazonium salts were treated in ethanol with sodium acetate and approximated amounts of active methylene material acetylacetone to provide compound (9) (Scheme 4). Cyclization of compound (9) with urea and thiourea, under ultrasonic conditions at 60°C leads to the formation of new pyrimidine derivatives containing urea and thiourea skeleton (10) and (11). IR spectra demonstrated the structure of (10) by the removal of the typical bands of NH_2 and the emergence of strong band in the 3462 cm-1, attributable to NH groups stretching, a band of the C=O groups at 1726 cm-^{1} for (pyrimidin-2-one), and C-H aliphatic at 2926-2834 cm^{-1}. The ^{1}HNMR spectra, which gave strong support for the pyrimidine structure, also indicated structure (10). There were 3NH groups

(14.57, 12.57, and 11.79), (8.69 - 7.03) for (1H pyrimidine + 4H, Ar-H), and 3CH_3 groups (2.62, 2.52, and 2.47).

The lack of acetyl C=O and carboxylic acid ester groups, as well as the presence of one methyl group at 14.42 ppm, two C=O for pyrimidine at 164.41, 163.32, and 156.83-115.98 for pyrimidine (6C-Ar + 4C - pyrimidine), was shown by ^{13}C NMR analysis of compound (10) (Scheme 4).



Scheme 4: Synthesis steps for diazotizing compound 7 then prepare compounds 10 and 11.

The thiazine derivatives (11) were determined using FT-IR, 1H-NMR, and ^{13}C-NMR. The general feature of IR spectrum of derivative (11) consists of presence of band at (3490 - 3034) cm^{-1} revealed a characteristic band for NH_2 and 2NH, 2879-2771 (C-H aliph. Str. cm^{-1}), and C=O at 1670 cm^{-1}. The ^1H NMR (400 MHz, $CDCl_3$) spectrum of compound (11) revealed the absence of $COCH_3$, as well as the presence of two broad singlets at 4.43 for NH_2, 11.79, 9.36 (2NH), 8.69 -7.01 (4H, Ar-H, + 3H- Pyrimidine) and 1.46, 2.36, 2.78, for (CH_3) groups. The ^{13}C NMR (101 MHz, CDCl3) spectra likewise suggested structure 11 providing solid support for the thiazine and pyrimidine structures. (Scheme 4)

2.5.3 Biological activity

2.5.4 Assay of in Vitro Antimicrobial Activity

Newly synthesized heterocyclic compounds containing sulfadiazine moiety derivatives were tested for in vitro antibacterial effectiveness towards fungal strains (C. albicans), Gram-negative bacterial strains (E. coli), and Gram-positive bacterial strains (S. aureus) using the tube dilution technique. Table 2 shows the MIC values for the compounds (6a, 6b, 9) that process the least MIC and inhibit E. coli growth at 200 µg/ml. Compounds 3, 5, 7,10, and 11 inhibit E. coli growth at 600 µg/ml, while compounds 4 and 6 inhibit growth at 1000 µg/ml.

Compounds (6a, 6b, 7,11) displayed substantial antibacterial activity against S. aureus at 200 µg/ml, whereas compounds (3,4,5,6,9,10) suppressed growth at 600 µg/ml, according to the antibacterial activity screening findings. Table 2 shows the MIC data for the compounds (3, 6b, 9,11) that process the least MIC and inhibit fungal strains (C. albicans) at 200 g/ml. Compounds (4, 5, 6a, 7, 10) inhibit C. albicans growth at 600 µg/ml, where compound (6) inhibit growth at 1000 µg/ml and inhibit fungal strain formation (C. albicans). Taking into account the antimicrobial findings of produced sulfonyl or sulfonamide-based derivatives with potential antibacterial capabilities against a variety of Gram-positive and Gram-negative bacteria's, and investigating its many structure-activity relationship (SAR) aspects. (El-Gaby et al., 2020, Hassan, 2019).

Table 1: Preparation of test sample.

Stock solution 4000µg/mL (0.02g in 5mL DMSO)				
NO.	Conc. (µg/mL)	Chemical Volume (µl)	Mueller Hinton broth volume (µl)	Bacterial suspension volume (µl)
1	200	12.5	187.5	50
2	600	37.5	162.5	50
3	1000	62.5	137.5	50

Table 2: Determination of minimum inhibitory concentration (MIC) of the most active newly synthesized compounds.

	Minimum Inhibitory Concentration (MIC µg/mL)		
Compound No.	G(+ ve) bacteria	G(-ve) bacteria	Fungi
	S. aureus	*E. coli*	*C.albicans*
3	600	600	200
4	600	1000	600
5	600	600	600
6	600	1000	1000
6a	200	200	600
6b	200	200	200
7	200	600	600
9	600	200	200
10	600	600	600
11	200	600	200
CIP 5µg/mL	5		-
FLU50µg/ mL	-		50

*The MIC is the minimal antibacterial agent availability that prevents the development of any organism.

Conclusion

Pyridazine, pyrazolines, thiazole, pyrimidine, and thiazine derivatives bearing a sulfonamide moiety were synthesized using both conventional and environmentally friendly ultrasonic sonication irradiation techniques, aligning with Green Chemistry principles. The structures of these compounds were elucidated through analysis of their IR, ^{1}H-NMR, and ^{13}C-NMR spectra. Subsequently, their antibacterial properties were investigated. Upon screening the synthesized chemicals, significant antimicrobial activities were observed, particularly with compounds (6a, 6b, 9, and 11). The minimum inhibitory concentration (MIC) for these compounds was found to be growth at 200 µg/ml, highlighting their potential as effective antimicrobial agents.

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