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Design And Synthesis Of Novel Heterocyclic Hybrids Bearing Thiazole, Thiazolidinone, And Piperazine And Their Evaluation As Antimicrobial And Antioxidant Agent

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Abstract
A series of novel heterocyclic hybrids based on pharmacophores such as Thiazole, Piperazin-4-(P-Chlorophenyl)-Sulfonamide, and Thiazolidin-4-one substituted at the 5th position by different aromatic aldehydes were synthesized (compound 8 a-j) and their structure was established with the aid of spectroscopic techniques such as FTIR, ^{13}C NMR, ^{1}H NMR, Mass, etc. In-vitro antibacterial, antifungal, and antioxidant activities of the synthesized compounds 8 a-j have been evaluated using ampicillin, Nystatin, and Ascorbic acid as the reference standards respectively. Except for compound 8C, all compounds have revealed comparable and better antibacterial and antifungal activity displaying similar MIC values as the reference standard used. Moreover, compound ***8e (125µg/ml)*** *has exhibited two-fold activity than the reference standard* ***(250µg/ml)*** *against gram – ive bacteria i.e. Proteus vulgaris. Although antioxidant activities are seen as ordinary compared to the reference standard, interestingly compounds* ***8d*** *and* ***8h*** *have displayed multi-target inhibitory action against different microbial as well as DPPH free radical, making it a possible choice as a single drug for the cure of multiple ailments.*

Keywords*: Thiazole, Thiazolidin-4-one, Piperazine, Antimicrobial, Antioxidant*

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I. Introduction

Microorganisms are naturally empowered to become resistant to drugs, and the past has given evidence of the same for instance, penicillin resistance bacteria, Staphylococcus aureus, Streptococcus pneumoniae, and Mycobacterium tuberculosis viz. [1]. Reckless, redundant, and inadequate intake of antimicrobial drugs for treating any infections, consequently surge the possibility of survival of a very small part of microbes and multiplies themselves to become the dominant form of that species and resistant to the drugs used previously to kill them [2]. Thus, Antimicrobial Resistance (AMR) is a disorder that needs to be well thought out to continue our chosen lifestyles and prevent misery. It has been alarmed by many healthcare organizations and WHO, which predicts that by the year 2050 about ten million people per year couldcause death due to AMR [3-5].

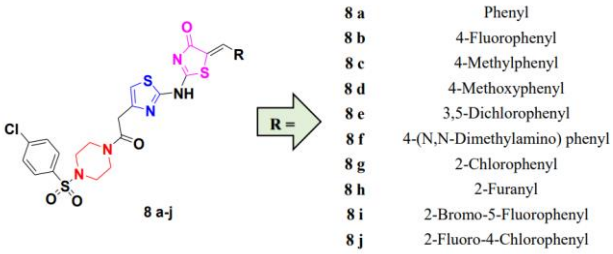
The past and present situations impacted our life and economy as well, inculcating us to find out new substitute drugs for the treatment of diseases that may occur in the future due to unprecedented infections which are resistant to the existing limited drugs [6]. In brief, global attention and stewardship are required for the discovery of new drugs due to the untimely outbreak of new diseases, drug resistance, lengthy drug approval process, and the high cost of the existing limited drugs. Thus, screening of novel Antimicrobial drugs has got a significant place in the field of medicinal chemistry to prevent such a calamity that doesn't yet exist and they're years away from patients [7-11].

Furthermore, presently we are fenced with other multiple critical disorders such as cancer, autoimmune disorders, aging, cardiovascular, and neurodegenerative and we are prone to get infected with these disorders owing to the aberration of free radical's oxidative stress which may be triggered as a result of dearth of antioxidant enzymes which have been synthesized in our body and also obtained from food or supplements rich in antioxidant properties [12-13]. Generally, reactive oxygen species (ROS) are free radicals such as Hydrogen peroxide (H_2O_2), Oxygen ($O_2^{\cdot}$), hydroxyl (•OH), singlet oxygen ($^1O_2$), etc. are metabolic by-products generated by our biological system [14-15]. Although natural resources and enzymatic oxidants are available to control excess oxidative stress, alternatives such as non-enzymatic antioxidants for this issue need to be discovered. Thus, antioxidants drugs or free radical scavengers are the need of the time, and maybe in the future, they shall be used as an effective

therapeutic approach for the treatment of cancer and other diseases [16].

Consequently, exploration for new lead compounds bearing antimicrobial and antioxidant activities with fewer adverse effects is the major challenge in drug discovery to increase longer life expectancy [17]. Heterocyclic compounds always have been a crucial part of the development of the drug in the interest of curing different diseases due to their high propensity for biological targets [18-19]. In our present study,we focused on a few active heterocyclic pharmacophores which have been studied widely for different biological activities. Heterocyclic Hybrid compounds as shown in fig.1 bearing Thiazole, 5-ene-4-Thiazolidinone, and Piperazin-4-(p-Chloro phenyl) sulfonamide have been synthesized and evaluated for their antimicrobial and Antioxidant activities. Thiazole and 5-ene-4-Thiazolidinone have got a unique place in medicinal chemistry due to their wide

Fig. 1: Synthesized Heterocyclic hybrid compound containing different pharmacophores



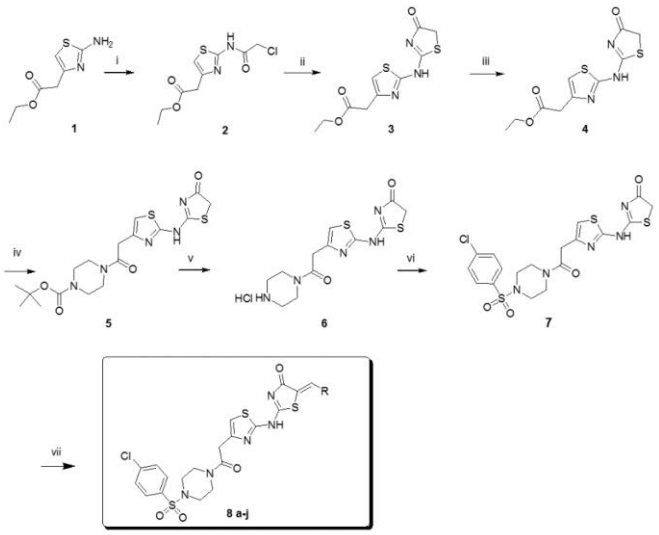
Range of biological activities [20-21]. Hybrid molecules obtained from thiazole, 5-ene-4-thiazolidinones have been studied extensively for instance antimicrobial [22-28], anti-inflammatory [29], anticancer [30], antidiabetic [31], antioxidant [32], antiviral [33], tyrosinase inhibitor [34], etc.

In addition to this, piperazine pharmacophore has been selected and introduced in our study based on a plethora of its biological activity [35]. The presence of the piperazine sulfonamide scaffold has exhibited enhancement of the antimicrobial [36] and antidiabetic activity [37]. 4-chloro phenyl sulphonyl chloride was used to prepare piperazinyl sulfonamide and the same has been chosen due to its proven effect on biological activity enhancement [38].

II. Materials and Methods

All chemicals such as Thiourea, Chloro ethyl acetoacetate, Sodium hydroxide, Boc-anhydride, Boc-Piperazine, 4-Chloro phenyl sulphonyl chloride, Di-isopropyl ethyl amine, Triethyl Amine, and solvents such as ethanol, MDC, DMF, Ethyl acetate, n-Hexane were obtained from Sigma-Aldrich, Avra and used without purification. Melting points were taken using the melting point apparatus. Infrared spectra were recorded on a Bruker spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained from a Bruker 500 MHz spectrometer. Biological activity evaluation was done at Aster analytics research institute. The antibacterial and antifungal activities were accomplished by using the Microbroth dilution method against Ampicillin and Nystatin as a standard. Antioxidant activity was accomplished by using the DPPH method against the Ascorbic acid standard.

Chemistry



Scheme-1: i) Chloroacetyl chloride, Triethyl amine, MDC, ii) Ammonium Thiocyanate, ethanol, iii) NaOH, Water: Ethanol, iv) Boc- Piperazine, TEA, and MDC, v) Dioxane HCl, vi) 4-Chloro Phenyl Sulfonyl chloride, TEA, MDC, vii) Sodium Acetate, Substituted aromatic aldehyde, ethanol.

Novel series of heterocyclic hybrids have been synthesized by the multi-step synthesis protocol as described in Scheme 1. Ethyl 2-(2-aminothiazol-4-yl) acetate (Compound 1) have synthesized by using Thiourea and ethyl Chloro acetoacetate to give the formation of (Ethyl 2-(2-(2-chloroacetamido) thiazol-4-yl) acetate (Compound 2) [39]. The reaction of compound 2 with Chloroacetyl chloride followed by ammonium Thiocyanate resulted in cyclization, forming another active scaffold i.e. Thiazolidin-4-one to afford Compound 3. It is then subjected to alkaline hydrolysis for conversion of ester to acid (Compound 4) which is further on reaction with Boc-protected Piperazine in presence of a base such as Triethyl amine resulting in the introduction of another active pharmacophore and gives a hybrid compound of three pharmacophores i.e. Compound 5. It is further reacting under strongly acidic conditions favors the removal of Boc protection (Compound 6). 4-Chloro-phenyl sulphonyl chloride is allowed to react with compound 6 to yield Piperazinyl sulfonamide (Compound 7). Finally, the Knoevenagel condensation reaction has been carried out by using different substituted aromatic aldehydes in presence of Sodium acetate in acetic acid as a solvent to convert the methylene group on the Thiazolidinone ring to the substituted benzylidene derivatives.

Chemical Procedure

Preparation of Ethyl 2-(2-(2-chloroacetamido) thiazol-4-yl) acetate (Compound 2): To the clear solution of Compound-1 (10.0 gm, 1.0 equivalent) in 200 ml of DCM, DIPEA (3.0 equivalents) was added at 0-5°C. Chloroacetyl chloride (1.2 equivalents) was added slowly to the clear solution at a maintained temperature. The reaction mixture was then maintained at room temperature and reaction progress was monitored by TLC. After 4 hours the reaction mixture was poured on water and an organic layer was collected. DCM layer was then washed with an aqueous sodium bicarbonate solution, Brine solution, and Water successively. Finally, the organic layer was dried over sodium sulfate and concentrated to obtain crude material which was purified by column chromatography (Silica 100-200 mesh) using 3-4 % Methanol in DCM as mobile phase to afford 11.0 gm of Compound-2 as a light brown solid. (78.02 % yield), MP = 194-195°C; MS (ESI) m/z calculated for $C_9H_{11}ClN_2O_3S$: $[M + H]^+$ 263.02, found 263.01.

Preparation of Ethyl 2-(2-(4, 5-dihydro-4-oxothiazol-2-ylamino) thiazol-4-yl) acetate (Compound 3): To the clear solution of compound-2 (5.5 g, 1.0 equivalent) in Ethanol (80 ml), Potassium Thiocyanate (3.05 g, 1.5 equivalent) was added and then stirred at reflux temperature for 8 hrs. Reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure till precipitation. The precipitated product was then filtered and washed with a 1:1 mixture of Water: Ethanol to obtain 5.3 gm of a light brown solid which further on slurry with n-Hexane and drying afforded 4.65 gm of compound 3 as a Light pink solid. (77.58 % yield), MP = 214-215°C; MS (ESI) m/z calculated for $C_{10}H_{11}N_3O_3S_2$: [M + H] + 286.02, found 286.01.

Preparation of 2-(2-(4, 5-dihydro-4-oxothiazol-2-ylamino) thiazol-4-yl) acetic acid (Compound 4):

To the stirred solution of compound 3 (3.8 gm, 1.0 equivalent) in Tetrahydrofuran (10.0 ml) and Methanol (5.0 ml), Lithium hydroxide (2.0 equivalent) was added and stirred at room temperature for 12 hrs. Monitored the reaction by TLC and after complete conversion, the reaction mixture was concentrated under reduced pressure, and to the residue, water (25 ml) was added. The pH of the reaction mixture was adjusted to 2-3 using a 2 N HCl solution to get the precipitation. The precipitated product was then filtered and washed with water followed by drying to afford 2.50 gm of compound 4 as an off-white solid. (73.09 % yield), MP = 254-255°C; MS (ESI) m/z calculated for $C_8H_7N_3O_3S_2$: [M + H] + 257.99, found 257.96.

Preparation of 2-(4-(2-oxo-2-(Boc-piperazin-1-yl) ethyl) thiazol-2-ylamino) thiazol-4(5H)-one(Compound 5): To a stirred solution of Compound 4 (2.5 g, 1eq) in DMF (30.00 mL), HATU (1.5 equivalent) and DIPEA (3.0 equivalent) were added at 0°C. The reaction mixture was stirred at room temperature for 5 minutes followed by the addition of Boc-Piperazine (2.38 gm, 1.1 equivalent). The reaction mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. After complete conversion, the reaction mixture was poured into ice-cold water (100.00 mL). The precipitated solid was filtered and dried to afford 4.9 gm of crude product which is on further slurry in n-Hexane followed by filtration and drying afforded 3.22 gm of compound 5 as an off-white solid. (78.09 % yield), MP = 174-175°C; MS (ESI) m/z calculated for $C_{17}H_{23}N_5O_4S_2$: [M + H] + 426.12, found 426.10.

Preparation of 2-(4-(2-oxo-2-(piperazin-1-yl) ethyl) thiazol-2-ylamino) thiazol-4-(5H)-one hydrochloride (Compound 6): Compound 5 (4.5 g, 1.0 equivalent) was dissolved in 4 M 1, 4-Dioxane: HCl (70.00 mL) and the reaction mixture was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC. After completion of starting material reaction mixture was concentrated under reduced pressure to afford 4.0 gm of an off-white semisolid. The obtained residue was stirred by adding n-Hexane (25.00 mL) and filtered followed by drying to afford 2.93 gm of compound 6 as an off-white white solid. (76.60 % yield),

MP = 159-160°C; MS (ESI) m/z calculated for $C_{12}H_{15}N_5O_2S_2$: [M + H] + 326.07, found 326.07.

Preparation of 2-(4-(2-oxo-2-(piperazin-1-{4-Chloro phenyl sulfonyl}) ethyl) thiazol-2-ylamino)thiazol-4(5H)-one (Compound 7): To a stirred solution of compound 6 (6.5 g, 1 equivalent) in DMF (30.00 mL), DIPEA (3 equivalent) was added. The reaction mixture was stirred at room temperature for 5 minutes followed by the addition of 4-Chloro benzene sulfonyl chloride (1.1 equivalent). The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by TLC. After 12 hrs. the reaction mixture was poured on ice-cold water (50.00 mL), to obtain the precipitation. The precipitated solid was filtered and washed with cold water to afford 5 g crude material which was further purified by column chromatography using silica 100-200 mesh and 70% ethyl acetate in hexane to afford 8.17 gm of Compound 7 as an off-white solid. (82.06 % yield), MP = 159-160°C; MS (ESI) m/z calculated for $C_{18}H_{18}ClN_5O_4S_3$: [M + H] $^+$ 500.02, found 500.02.

General procedure for Preparation of (Compound 8 a-j): To the stirred solution of Compound 7 in Acetic acid (5.0 ml), Sodium Acetate (6 equivalents) and Aromatic aldehydes (2.0 equivalents) were added the following by heating the reaction mixture to reflux and monitored the reaction by TLC. After 8 to 10 hrs. reaction mixture was cooled to room temperature and poured into ice water to get precipitation. Obtained precipitated crude was then filtered and washed with water followed by drying to give crude material, which is purified further by using a 1:1 mixture of Ethyl acetate and n-Hexane and finally by recrystallization using ethanol to obtain final compound 8a-j as a Yellow to light brown colored solid.

Compound 8 a: Light brown solid, (75% yield); MP = 285-286°C; IR (Nujol): cm −1 = 3030, 1723, 1574, 1255, 1153, 1094, 1017, 770, 680, 598, 528; 1H-NMR (500 MHz, DMSO): δ 12.82 (s, 1H, NH), 7.98 (s, 1H), 7.50-7.72 (m, 10 H), 4.21 (m, 2H), 3.77 (m, 2H), 3.33 (m, 2H), 3.08, 3.01 (m, 4H); 13C-NMR (125 MHz, DMSO): δ; 168.04, 167.30, 161.54, 156.74, 146.71, 138.85, 134.61, 133.81, 132.84, 130.93, 130.50, 130.12, 129.81, 129.78, 124.60, 124.49, 46.71, 46.25, 45.37, 42.08, 40.33, 40.16, 39.99, 39.83, 39.66; MS (ESI) m/z calculated for $C_{25}H_{22}ClN_5O_4S_3$: [M + H]$^+$ 588.05, found 588.03.

Compound 8 b: Light orange solid, (73 % yield); MP = 260-262°C; IR (Nujol): cm^{-1} = 3030, 1724, 1576, 1258, 1152, 1096, 1018, 776, 690, 599, 523; 1H-NMR (500 MHz, DMSO): δ 12.86 (s, 1H, NH), 7.98 (s, 1H), 7.77 (s, 1H), 7.65-7.75 (m, 8 H), 4.19 (m, 2H), 3.88 (m, 2H), 3.33 (m, 2H), 3.02- 3.08 (m, 4H) ; 13C-NMR (125 MHz, DMSO): δ; 167.94, 167.23, 161.53, 156.35, 146.76, 138.85, 135.64, 134.62, 134.54, 132.65, 132.10, 131.46, 130.13, 130.09, 129.91, 129.80, 125.11, 124.60, 46.76, 46.14, 45.37, 41.95, 40.33, 40.16, 39.99, 39.83, 39.66; MS (ESI) m/z calculated for $C_{25}H_{21}ClN_5O_4S_3$: [M + H]$^+$ 606.04, found 606.03.

Compound 8 c: Light brown solid, (68% yield); MP = 272-273°C; IR (Nujol): cm −1 = 3028, 1727, 1582, 1260, 1155, 1097, 1020, 775, 691, 600, 525; 1H-NMR (500 MHz, DMSO): δ 12.73 (s, 1H, NH), 7.98 (s, 1H), 7.62-7.74 (m, 7 H), 7.17 (d, 2H, J= 5 Hz), 4.25 (m, 2H), 3.78 (m, 2H), 3.33 (m, 2H), 3.02-3.10 (m, 4H), 2.4 (s, 3H); 13C-NMR (125 MHz, DMSO): δ; 168.13, 167.48, 161.59, 161.46, 156.85, 146.72, 138.86, 134.56, 132.94, 132.62, 130.11, 129.81, 126.20, 124.55, 121.08, 115.43, 56.00, 46.76, 46.21, 45.37, 41.95, 40.33, 40.16, 39.99, 39.83, 39.66, 21.55; MS (ESI) m/z calculated for $C_{26}H_{24}ClN_5O_4S_3$: [M + H]$^+$ 602.07, found 602.09.

Compound 8 d: Light brown solid, (69% yield); MP = 271–272°C; IR (Nujol): cm −1 = 3020, 1721, 1576, 1260, 1150, 1093, 1018, 775, 685, 591, 520; 1H-NMR (500 MHz, DMSO): δ 12.73 (s, 1H, NH), 7.98 (s, 1H), 7.62-7.74 (m, 7 H), 7.17 (d, 2H, J= 5 Hz), 4.25 (m, 2H), 3.87 (s, 3H), 3.78 (m, 2H), 3.33 (m, 2H), 3.01-3.10 (m, 4H),; 13C-NMR (125 MHz, DMSO): δ; 168.13, 167.48, 161.59, 161.46, 156.85, 146.72, 138.86, 134.56, 132.94, 132.62, 130.11, 129.81, 126.20, 124.55, 121.08, 115.43, 56.00, 46.76, 46.21, 45.37, 41.95, 40.33, 40.16, 39.99, 39.83, 39.66; MS (ESI) m/z calculated for $C_{26}H_{24}ClN_5O_5S_3$: [M + H]$^+$ 618.06, found 618.05.

Compound 8 e: Light red solid, (70.0 % yield); MP = 255–256°C; IR (Nujol): cm^{-1} = 3015, 1720, 1570, 1275, 1152, 1091, 1010, 775, 680, 593, 525; 1H-NMR (500 MHz, DMSO): δ 12.98 (s, 1H, NH), 7.98 (s, 1H), 7.88 (s, 1H), 7.82 (s, 1H), 7.68 (s, 6 H), 4.10 (m, 2H), 3.74 (m, 2H), 3.33 (m, 2H), 3.02-3.04 (m, 4H); 13C-NMR (125 MHz, DMSO): δ; 167.81, 166.90, 161.50, 156.01, 146.73, 138.83, 136.20, 135.79, 134.63, 130.76, 130.57, 130.13, 130.08, 129.78, 128.73, 126.59, 124.69, 46.76, 46.21, 45.37, 41.91, 40.16, 40.00, 39.83; MS (ESI) m/z calculated for $C_{25}H_{20}Cl_3N_5O_4S_3$: [M + H]$^+$ 655.97, found 655.96.

Compound 8 f: Light brown solid, (73% yield); MP = 275-276°C; IR (Nujol): cm^{-1} = 3025, 1720, 1580, 1260, 1158, 1097, 1025, 770, 675, 586, 525; 1H-NMR (500 MHz, DMSO): δ 12.56 (s, 1H, NH), 7.70-7.72 (m, 5H), 7.48-7.54 (m, 2H), 6.88-6.90 (d, 2H), 4.33 (m, 2H), 3.78 (m, 2H), 3.33 (m, 2H), 3.06, 3.02 (m, 10 H); 13C-NMR (125 MHz, DMSO): δ; 168.32, 167.58, 161.37, 157.12, 152.07, 146.68, 139.01, 138.86, 134.51, 134.15, 132.77, 130.12, 129.94, 129.82, 129.78, 124.37, 120.41, 116.23, 112.53, 111.53, 46.82, 46.23, 45.37, 41.97, 40.50, 40.42, 40.33, 40.25, 40.16, 40.09, 40.00, 39.92, 39.83, 39.66, 39.49; MS (ESI) m/z calculated for $C_{27}H_{27}ClN_6O_4S_3$: [M + H]$^+$ 631.09, found 631.09.

Compound 8 g: Light brown solid, (66.0% yield); MP = 289-290°C; IR (Nujol): cm^{-1} = 3030, 1724, 1576, 1258, 1152, 1096, 1018, 776, 690, 599, 523; 1H-NMR (500 MHz, DMSO): δ 12.86 (s, 1H, NH), 7.98 (s, 1H), 7.77 (s, 1H), 7.65-7.75 (m, 8 H), 4.19 (m, 2H), 3.88 (m, 2H), 3.33 (m, 2H), 3.02-3.08 (m, 4H) ; 13C-NMR (125 MHz, DMSO): δ; 167.94, 167.23, 161.53, 156.35, 146.76, 138.85, 135.64, 134.62, 134.54, 132.65, 132.10, 131.46, 130.13, 130.09, 129.91, 129.80, 125.11, 124.60, 46.76, 46.14, 45.37, 41.95, 40.33, 40.16, 39.99, 39.83, 39.66;

MS (ESI) m/z calculated for $C_{25}H_{21}Cl_2N_5O_4S_3$: $[M + H]^+$ 622.01, found 622.01.

Compound 8 h: Light brown solid, (59% yield); MP = 281–282°C; IR (Nujol): cm $^{-1}$ = 3010, 1735, 1580, 1255, 1150, 1097, 1020, 780, 686, 595, 529; 1H-NMR (500 MHz, DMSO): δ 13.00 (s, 1H, NH), 7.96 (s, 1H), 7.89 (dd, 1H, J=10 Hz), 7.65-7.74 (m, 1H), 7.44 (dd, 1H, J= 10 Hz), 7.36 (m, 1H, J = 5 MHz), 7.345 (ddd, 1H, 15 Hz), 4.21 (m, 2H), 3.77 (m, 2H), 3.33 (m, 2H), 2.95-3.01 (m, 4H); 13C-NMR (125 MHz, DMSO): δ; 168.04, 167.30, 161.54, 156.74, 146.71, 138.85, 134.61, 133.81, 132.84, 130.93, 130.50, 130.12, 129.81, 129.78, 124.60, 124.49, 46.71, 46.25, 45.37, 42.08, 40.33, 40.16, 39.99, 39.83, 39.66; MS (ESI) m/z calculated for $C_{23}H_{20}ClN_5O_4S_3$: $[M + H]^+$ 578.08, found 578.09.

Compound 8 i: Off white solid, (70 % yield); MP = 244–245°C; IR (Nujol): cm^{-1} = 3015, 1720, 1570, 1275, 1152, 1091, 1010, 775, 680, 593, 525; 1H-NMR (500 MHz, DMSO): δ 12.98 (s, 1H, NH), 7.98 (s, 1H), 7.88 (s, 1H), 7.82 (s, 1H), 7.68 (s, 6 H), 4.10 (m, 2H), 3.74 (m, 2H), 3.33 (m, 2H), 3.04, 3.02 (mm, 4H); 13C-NMR (125 MHz, DMSO): δ; 167.81, 166.90, 161.50, 156.01, 146.73, 138.83, 136.20, 135.79, 134.63, 130.76, 130.57, 130.13, 130.08, 129.78, 128.73, 126.59, 124.69, 46.76, 46.21, 45.37, 41.91, 40.16, 40.00, 39.83; MS (ESI) m/z calculated for $C_{25}H_{22}ClN_5O_4S_3$: $[M + H]^+$ 683.95, found 683.93.

Compound 8 j: Light yellow solid, (62% yield); MP = 259-260°C; IR (Nujol): cm^{-1} = 3015, 1720, 1570, 1275, 1152, 1091, 1010, 775, 680, 593, 525; 1H-NMR (500 MHz, DMSO): δ 12.98 (s, 1H, NH), 7.98 (s, 1H), 7.88 (s, 1H), 7.82 (s, 1H), 7.68 (s, 6 H), 4.10 (m, 2H), 3.74 (m, 2H), 3.33 (m, 2H), 3.02-3.04 (m, 4H); 13C-NMR (125 MHz, DMSO): δ; 167.81, 166.90, 161.50, 156.01, 146.73, 138.83, 136.20, 135.79, 134.63, 130.76, 130.57, 130.13, 130.08, 129.78, 128.73, 126.59, 124.69, 46.76, 46.21, 45.37, 41.91, 40.16, 40.00, 39.83; MS (ESI) m/z calculated for $C_{25}H_{20}Cl_2N_5O_4S_3$: $[M + H]^+$ 640.00, found 640.01.

In-vitro Antibacterial and antifungal activity by Microbroth dilution method:

The MICs for *Bacillus subtilis NCIM 2063, Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2065, Proteus vulgaris NCIM 2813, Aspergillus Niger NCIM 501,* and *Candida albicans NCIM 3471* microorganisms were determined. Briefly, testing was performed in sterile 96-well microtiter plates. The MIC values of synthesized products (8a-j) were determined based on a micro-broth dilution method in 96 multi-well microtiter plates with slight modifications. A stock solution of resazurin sodium salt powder (Sigma Aldrich) was prepared at 0.02% (w/v) in distilled water and stored at 4 °C for up to 1 week.

Procedure

A volume of 100 µl of synthesized compound (8a-j) in 10% (v/v) DMSO (usually a stock concentration of 1 mg/ml for synthesized compound) was added into the first row of the plate. 50 µl of nutrient broth and 50 µl of normal saline were added to each well of the plate. Serial dilutions were performed using a multichannel pipette such that each well had a total of l00 µl of the test material in serially descending concentrations. 10 µl of resazurin indicator solution was added to each well. Finally, 0.5 McFarland standard microbial suspension of l0 µl of bacterial and fungal suspension was added to each well to achieve a concentration of 1.5×108CFU/ml (for bacteria) and 0.5-2.5×103 yeast cells or spores/ml (fungi) [40-42].

Each plate had a column with Ampicillin as the positive control and DMSO as the negative control for bacteria and Nystatin as the positive control and DMSO as the negative control in the case of Fungi. The plates were prepared in triplicates and placed in an incubator set at 37 °C for 18-24 hr. for bacteria and 25 °C for 48 hr. for Fungi.

Final concentrations of the compounds in the liquid media ranged from 1000 to 0.0038 µg/ml. Microbial suspensions were added per each well containing broth and various concentrations of the examined compounds. After incubation, the MIC was determined spectrophotometrically as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, sterile, and growth controls were carried out. The media with no tested substances were used also as controls. Any color changes from purple to pink or to colorless indicated the growth of microbes. The lowest concentration at which no color change occurred was taken as the MIC value of the synthesized compound [43].

DPPH Assay method for the evaluation of Antioxidant activity

The molecule 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is characterized as a stable free radical by the delocalization of the free electron around the molecule so that the molecule does not combine to form a stable molecule. The stability of the electron also gives deep violet color, characterized by an absorption band in ethanol solution at about 517 nm. When a solution of DPPH is mixed with that of a reacting species that can donate a hydrogen atom, this gives rise to the reduced form with the loss of this violet color. The percentage DPPH inhibition activity of synthesized compounds (8a-j) and the ascorbic acid are measured at different concentrations between 25-400 µg/ml and reported [44-45].

III. Result

In-vitro Antibacterial and antifungal Activity

Novel series of Thiazole/ Thiazolidinone/Piperazinyl sulfonamide 8 a-j were evaluated for antibacterial activity by using the micro broth dilution method against a set of Gram-positive (Bacillus subtilis NCIM 2063 and Staphylococcus aureus NCIM 2079) and gram-negative (Escherichia coli NCIM 2065; PV: Proteus vulgaris NCIM 2813) bacterial microorganisms and ampicillin used as a reference standard.

Table 1: Antibacterial and antifungal activities of compound 8 a-j against *BS:* Bacillus subtilis NCIM 2063; *SA*: Staphylococcus aureus NCIM 2079, *EC*: Escherichia coli NCIM 2065; *PV*: Proteus vulgaris NCIM 2813, *AN*: Aspergillus Niger NCIM 501; and *CA*: Candida albicans NCIM 3471 microorganisms, standard: Ampicillin (For gram +ive and –ive bacterial microorganisms); Nystatin (for fungal microorganisms).

Compound	*Minimum Inhibitory Concentration (MIC) in µg/ml*					
	Gram +ive Bacteria		Gram - ive Bacteria		Fungal Microorganism	
	BS	*SA*	*EC*	*PV*	*AN*	*CA*
8 a	500	250	250	375	**250**	250
8 b	**125**	375	187.5	**187.5**	500	**125**
8 c	250	375	187.5	375	**187.5**	375
8 d	187.5	250	250	**250**	375	**125**
8 e	250	375	250	**125**	**187.5**	**187.5**
8 f	**125**	375	187.5	**250**	375	500
8 g	500	375	187.5	375	**187.5**	375
8 h	187.5	375	187.5	**187.5**	**187.5**	**187.5**
8 i	187.5	**187.5**	187.5	375	**250**	375
8 j	375	375	250	375	**250**	250
Standard	**125**	**187.5**	**125**	**250**	**250**	**187.5**

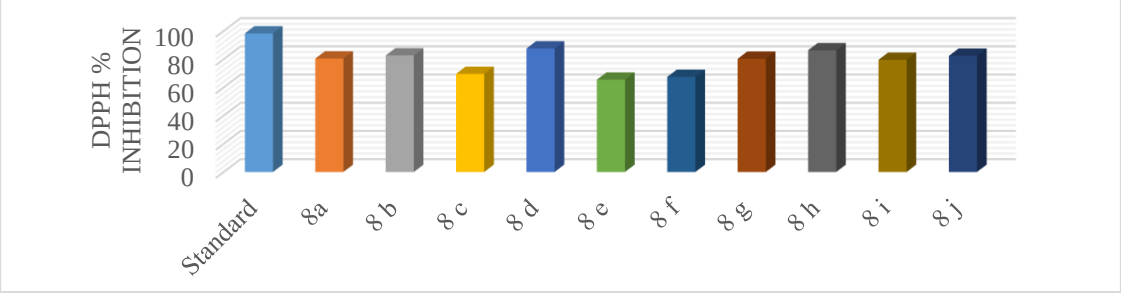
In-Vitro Antioxidant activity

The hybrid series prepared from Thiazole, Thiazolidinone, and Piperazinyl Sulphonamide in this study were tested for their in vitro antioxidant activity at several concentrations ranging from 25 to 400 μg/mL of compounds and standard as well by DPPH assay.

Table 2: Antioxidant activities of compound 8 a-j (Values are mean ± SEM of triplicate determinations)

Conc. µg/ml	*DPPH % Inhibition*										
	Standard	**8 a**	**8 b**	**8 c**	**8 d**	**8 e**	**8 f**	**8 g**	**8 h**	**8 i**	**8 j**
25	35.39	22.05	26.53	23.68	22.56	23.60	21.54	23.65	21.11	27.56	25.41
50	68.64	41.25	54.21	36.51	44.58	39.66	38.95	42.65	35.26	56.51	45.02
75	79.89	64.26	61.59	42.51	61.54	45.32	46.10	48.95	41.02	69.32	49.36
100	87.89	71.26	70.22	52.32	70.56	48.51	51.25	52.36	46.55	73.65	56.21
200	92.13	75.36	75.66	57.14	78.52	52.34	59.64	59.21	55.69	75.14	67.22
300	95.13	79.55	79.60	61.26	82.36	59.68	62.55	70.25	72.56	77.58	79.22
400	98.26	80.40	82.61	69.54	**87.55**	65.44	67.44	80.26	**86.32**	79.52	82.35

Fig. 2: Graphical representation of % inhibition of DPPH free radical at 400 µg/ml concentration.



IV. Discussion

In-vitro Antibacterial Activity:

According to the results obtained and shown in Table-1, it is observed that all compounds have displayed good to excellent antibacterial activity. It was noticed that most of the compounds in the series have shown better inhibition of Proteus vulgaris NCIM 2813 bacterial microorganisms. Among the series, compounds 8 b, 8 d, 8 e, 8 f, 8 h, and 8 i have shown almost similar and enhanced activity displaying lower MIC values than reference standards against Gram-positive and gram-negative bacterial microorganisms. It is observed that the presence of fluoro (8b; MIC = 125 *µg/ml*) and N, N-Dimethyl amino (8f; MIC = 125 *µg/ml*) substituent on the para position of the benzylidene ring have exhibited better activity for the inhibition of Bacillus subtilis NCIM 2063. Compound 8 i, bearing two halogens substituent Bromo and Fluoro at the 2^{nd} and 5^{th} position respectively on the benzylidene ring have displayed antibacterial activity against Staphylococcus aureus (MIC = 187.5 *µg/ml*). The inhibitory action of all the synthesized novel compounds against one of the chosen gram –ive bacterial microorganisms i.e. Escherichia coli was seen as ordinary as compared to a reference standard. Moreover, most of the compounds 8b, 8d, 8e, 8f, and 8h have shown better and similar activity as reference standard but compound 8e bearing benzylidene ring substituted with dichloride group at both Meta positions have displayed two fold active than the reference standard (MIC = 250 *µg/ml*) by representing half of the MIC value (125 *µg/ml*) required for the inhibition of Proteus vulgaris microorganism making it than standard.

In-vitro Antifungal activity:

In-vitro antifungal activity of compound **8 a-j** has been determined and displayed in table-1. It was summarized that given series of hybrid compounds have displayed good to better activity against Aspergillus Niger and Candida albicans selected fungal microorganisms. Except for **8b, 8d**, and **8f** all synthesized hybrid compounds have displayed similar and better activities than the reference standard against Aspergillus Niger fungal microorganisms. Moreover, some of the compounds have exhibited superior activity displaying less MIC values **(8b, 8d = 125 *µg/ml*)** and similar MIC values **(8e, 8h = 187.5 *µg/ml*)** as compared to the reference standard for the inhibition of Candida albicans microorganism.

In-Vitro Antioxidant activity:

It was clearly seen from the results shown in **Table 2** that the radical scavenging activity of the compounds was found to be dependent on a concentration manner. All compounds have displayed moderate to good activity against DPPH free radicals. Among studied hybrid compounds in this study, compounds **8d** and **8h** have displayed maximum DPPH % of inhibition and are comparable to the activity of the ascorbic acid used as a standard as shown in **Fig. 2**. The presence of methoxy substituent at the para position of the benzylidene ring and 2-furanylidine ring attached to the 4-Thiazolidinone ring have shown **87.55 %** and **86.32 %** of inhibition of free radical against the standard with **98.26 %** inhibition.

V. Conclusion

Prime motivation of the present study was to synthesize and screening of the novel hybrid compounds by applying simple chemistry to connect two or more active heterocyclic scaffolds against gram positive/ negative bacterial and fungal microorganisms along with antioxidant properties. From our study, it was established that almost all synthesized compounds comprises Thiazole, Thiazolidinone and Piperazine have displayed good to better in-vitro antibacterial and antifungal activities. On other hand, ordinary results have seen for antioxidant activity. It is concluded that, except compound c, all compounds in the series have revealed good to better antimicrobial activity representing the MIC values comparable to the reference standard, making it as an alternative drug in the future. Furthermore, Compound **8d** and **8h** has come out as a promising compound which has shown antibacterial, antifungal and antioxidant activity as well. Although, we aim to evaluate cytotoxicity of these lead compounds obtained from this series. Additionally, it can be also envisioned that these compounds can be further studied for other microbial microorganisms as well as some structural modifications such as use of different Piperazinyl Sulphonamide or aromatic aldehydes may express favorable activity and it would be helpful to discover promising compound.

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References

[1]. Ventola Cl. The Antibiotic Resistance Crisis: Part 1: Causes And Threats. P T. 2015; 40 (4); 277-283.
[2]. Cantas L, Shah Sq, Cavaco Lm, Et Al. A Brief Multi-Disciplinary Review On Antimicrobial Resistance In Medicine And Its Linkage

To The Global Environmental Microbiota. Front Microbiol. 2013; 4; 96. 10.3389/Fmicb.2013.00096.
[3]. Daniela Pires, Marlieke Elizabeth Adriana De Kraker, Ermira Tartari, Mohamed Abbas, Didier Pittet, 'Fight Antibiotic Resistance—It's In Your Hands': Call From The World Health Organization For 5th May 2017, Clinical Infectious Diseases, 2017; 64(12); 1780–1783. Https://Doi.Org/10.1093/Cid/Cix226.
[4]. O'neill J. Tackling Drug-Resistant Infections Globally: Final Report And Recommendations. London: Review On Antimicrobial Resistance, 2016.
[5]. Antimicrobial Resistance Collaborators. Global Burden Of Bacterial Antimicrobial Resistance In 2019: A Systematic Analysis Published Correction Appears In Lancet. 2022 ;400; (10358) 10.1016/S0140-6736(21)02724-0.
[6]. Nolte O. Antimicrobial Resistance In The 21st Century: A Multifaceted Challenge. Protein Pept Lett. 2014; 21(4); 330-335. 10.2174/0929866513206660106.
[7]. Miethke, M., Pieroni, M., Weber, T. Et Al. Towards The Sustainable Discovery And Development Of New Antibiotics. Nat Rev Chem. 2021; 5; 726–749. Https://Doi.Org/10.1038/S41570-021-00313-1.
[8]. Ribeiro Da Cunha B, Fonseca Lp, Calado Crc. Antibiotic Discovery: Where Have We Come From, Where Do We Go? Antibiotics (Basel) 2019; 8(2); 45. 10.3390/Antibiotics8020045.
[9]. Kevin Outterson Et Al, We Need New Drugs To Prevent The Looming Superbug Crisis. , Stat (2020), Https://Www.Statnews.Com/2020/08/12/We-Need-New Antimicrobials-To-Prevent-The-Next-Infectious-Disease-Crisis/.
[10]. Oliver Trevelyan Et Al, The Importance Of Discovering New Drugs, Https://Www.Azolifesciences.Com/Article/The-Importance-Of-Discovering-New Drugs.Aspx.
[11]. Fisher, M.C., Alastruey-Izquierdo, A., Berman, J. Et Al. Tackling The Emerging Threat Of Antifungal Resistance To Human Health. Nat Rev Microbiol 2022; 20; 557–571. Https://Doi.Org/10.1038/S41579-022-00720-1.
[12]. Waris G, Ahsan H. Reactive Oxygen Species: Role In The Development Of Cancer And Various Chronic Conditions. J Carcinog. 2006; 5; 14. 10.1186/1477-3163-5-14.
[13]. Evans Md, Dizdaroglu M, Cooke Ms. Oxidative Dna Damage And Disease: Induction, Repair And Significance. Mutat Res. 2004; 567(1);1-61;10.1016/J.Mrrev.2003.11.001.
[14]. Sato H, Shibata M, Shimizu T, Et Al. Differential Cellular Localization Of Antioxidant Enzymes In The Trigeminal Ganglion. Neuroscience. 2013; 248; 345-358. 10.1016/J.Neuroscience.2013.06.010.
[15]. Navarro-Yepes J, Zavala-Flores L, Anandhan A, Et Al. Antioxidant Gene Therapy Against Neuronal Cell Death. Pharmacol Ther. 2014; 142(2); 206-230. 10.1016/J.Pharmthera.2013.12.007.
[16]. Valko M, Izakovic M, Mazur M, Rhodes Cj, Telser J. Role Of Oxygen Radicals In Dna Damage And Cancer Incidence. Mol Cell Biochem. 2004; 266(1-2); 37-56. 10.1023/B:Mcbi.0000049134.69131.89.
[17]. Thyagarajan A, Sahu Rp. Potential Contributions Of Antioxidants To Cancer Therapy: Immunomodulation And Radiosensitization. Integr Cancer Ther. 2018; 17(2); 210-216. 10.1177/1534735416681639.
[18]. Jampilek J. Heterocycles In Medicinal Chemistry. Molecules. 2019; 24(21); 3839. 10.3390/Molecules24213839.
[19]. Gomtsyan, A. Heterocycles In Drugs And Drug Discovery. Chem Heterocycl Comp. 2012; 48; 7–10. Https://Doi.Org/10.1007/S10593-012-0960-Z.
[20]. Petrou A, Fesatidou M, Geronikaki A. Thiazole Ring-A Biologically Active Scaffold. Molecules. 2021; 26(11); 3166. 10.3390/Molecules26113166.
[21]. Kaminskyy D, Kryshchyshyn A, Lesyk R. 5-Ene-4-Thiazolidinones - An Efficient Tool In Medicinal Chemistry. Eur J Med Chem. 2017; 140; 542-594. 10.1016/J.Ejmech.2017.09.031.
[22]. Vicini P, Geronikaki A, Incerti M, Zani F, Dearden J, Hewitt M. 2-Heteroarylimino-5-Benzylidene-4-Thiazolidinones Analogues Of 2-Thiazolylimino-5-Benzylidene-4-Thiazolidinones With Antimicrobial Activity: Synthesis And Structure-Activity Relationship. Bioorg Med Chem. 2008; 16(7); 3714-3724. 10.1016/J.Bmc.2008.02.001.
[23]. Patel, R.V., Park, S.W. Catalytic N-Formylation For Synthesis Of 6-Substituted-2-Benzothiazolylimino-5-Piperazinyl-4-Thiazolidinone Antimicrobial Agents. Res Chem Intermed 2015; 41; 5599–5609. Https://Doi.Org/10.1007/S11164-014-1684-8.
[24]. Abo-Ashour Mf, Eldehna Wm, George Rf, Et Al. Novel Indole-Thiazolidinone Conjugates: Design, Synthesis And Whole-Cell Phenotypic Evaluation As A Novel Class Of Antimicrobial Agents. Eur J Med Chem. 2018; 160; 49-60.10.1016/J.Ejmech.2018.10.008.
[25]. Haroun M, Tratrat C, Tsolaki E, Geronikaki A. Thiazole-Based Thiazolidinones As Potent Antimicrobial Agents. Design, Synthesis And Biological Evaluation. Comb Chem High Throughput Screen. 2016; 19(1); 51-57.10.2174/1386207319666151203002348.
[26]. Kocabalkanli A, Ates O, Otük G. Synthesis Of Mannich Bases Of Some 2,5-Disubstituted 4-Thiazolidinones And Evaluation Of Their Antimicrobial Activities. Arch Pharm (Weinheim). 2001; 334(2); 35-39. 10.1002/1521-4184(200102)334:2<35::Aid-Ardp35>3.0.Co;2-4.
[27]. Omar K, Geronikaki A, Zoumpoulakis P, Et Al. Novel 4-Thiazolidinone Derivatives As Potential Antifungal And Antibacterial Drugs. Bioorg Med Chem. 2010; 18(1); 426-432. 10.1016/J.Bmc.2009.10.041.
[28]. Abdellatif Kr, Abdelgawad Ma, Elshemy Ha, Alsayed Ss. Design, Synthesis And Biological Screening Of New 4-Thiazolidinone Derivatives With Promising Cox-2 Selectivity, Anti-Inflammatory Activity And Gastric Safety Profile. Bioorg Chem. 2016; 64; 1-12. 10.1016/J.Bioorg.2015.11.001.
[29]. Apostolidis I, Liaras K, Geronikaki A, Et Al. Synthesis And Biological Evaluation Of Some 5-Arylidene-2-(1,3-Thiazol-2-Ylimino)-1,3-Thiazolidin-4-Ones As Dual Anti-Inflammatory/Antimicrobial Agents. Bioorg Med Chem. 2013; 21(2); 532-539. 10.1016/J.Bmc.2012.10.046.
[30]. Geronikaki A, Eleftheriou P, Vicini P, Alam I, Dixit A, Saxena Ak. 2-Thiazolylimino/Heteroarylimino-5-Arylidene-4-Thiazolidinones As New Agents With Shp-2 Inhibitory Action. J Med Chem. 2008; 51(17); 5221-5228. 10.1021/Jm8004306.
[31]. Khan S, Iqbal S, Khan M, Et Al. Design, Synthesis, In Silico Testing, And In Vitro Evaluation Of Thiazolidinone-Based Benzothiazole Derivatives As Inhibitors Of A-Amylase And A-Glucosidase. Pharmaceuticals (Basel). 2022; 15(10); 1164. 10.3390/Ph15101164.
[32]. Abu-Melha S. Synthesis, Modeling Study And Antioxidants Activity Of New Heterocycles Derived From 4-Antipyrinyl-2-Chloroacetamidothiazoles. Applied Sciences. 2018; 8(11); 2128. Https://Doi.Org/10.3390/App8112128.
[33]. Xiao M, Xu L, Lin D, Et Al. Design, Synthesis, And Bioassay Of 4-Thiazolinone Derivatives As Influenza Neuraminidase Inhibitors. Eur J Med Chem. 2021; 213; 113161. 10.1016/J.Ejmech.2021.113161.
[34]. Gawande Ss, Warangkar Sc, Bandgar Bp, Khobragade Cn. Synthesis Of New Heterocyclic Hybrids Based On Pyrazole And Thiazolidinone Scaffolds As Potent Inhibitors Of Tyrosinase. Bioorg Med Chem. 2013; 21(10); 2772-2777. 10.1016/J.Bmc.2012.12.053.
[35]. Sharma A, Wakode S, Fayaz F, Khasimbi S, Pottoo Fh, Kaur A. An Overview Of Piperazine Scaffold As Promising Nucleus For Different Therapeutic Targets. Curr Pharm Des. 2020; 26(35); 4373-4385. 10.2174/1381612826666200417154810.

[36]. Shinde, Ravindra & Gaikwad, Dattatray & Farooqui, Mazahar. (2020). Synthesis And Antimicrobial Activity Of 2-(4-(Benzodthiazol-5-Ylsulfonyl) Piperazine-1-Yl)-N-Substituted Acetamide Derivatives. Journal Of Heterocyclic Chemistry 2020; 57(11); Http://Dx.Doi.Org/10.1002/Jhet.4099

[37]. Murthy Vs, Tamboli Y, Krishna Vs, Et Al. Synthesis And Biological Evaluation Of Novel Benzhydrylpiperazine-Coupled Nitrobenzenesulfonamide Hybrids. Acs Omega. 2021; 6(14); 9731-9740. 10.1021/Acsomega.1c00369.

[38]. Jaydeo T. Kilbile, Yasinalli Tamboli, Suchita S. Gadekar, Imadul Islam, Claudiu T. Supuran, Suryakant B. Sapkal, An Insight Into The Biological Activity And Structure-Based Drug Design Attributes Of Sulfonylpiperazine Derivatives, Journal Of Molecular Structure, 2023; 1278; 134971. Https://Doi.Org/10.1016/J.Molstruc.2023.134971.

[39]. Xiang J, Wan Zk, Li Hq, Et Al. Piperazine Sulfonamides As Potent, Selective, And Orally Available 11beta-Hydroxysteroid Dehydrogenase Type 1 Inhibitors With Efficacy In The Rat Cortisone-Induced Hyperinsulinemia Model. J Med Chem. 2008; 51(14); 4068-4071. 10.1021/Jm8004948.

[40]. Obasi Ln, Oruma Us, Al-Swaidan Ia, Ramasami P, Ezeorah Cj, Ochonogor Ae. Synthesis, Characterization And Antibacterial Studies Of N-(Benzothiazol-2-Yl)-4-Chlorobenzenesulphonamide And Its Neodymium (Iii) And Thallium (Iii) Complexes. Molecules. 2017; 22(2); 153. 10.3390/Molecules22020153.

[41]. Gulnaza., R. "Evaluation Of Antimicrobial Activity Of Leaf And Stem Extracts Of Sidda Medicinal Plant Sida Cordata." . Int J Med Pharma Sci 2013; 3; 39-50.

[42]. Martin A, Camacho M, Portaels F, Palomino Jc. Resazurin Microtiter Assay Plate Testing Of Mycobacterium Tuberculosis Susceptibilities To Second-Line Drugs: Rapid, Simple, And Inexpensive Method. Antimicrob Agents Chemother. 2003; 47 (11); 3616-3619. 10.1128/Aac.47.11.3616-3619.2003.

[43]. National Committee For Clinical Laboratory Standards (Nccls) (1999) Performance Standards For Antimicrobial Susceptibility Testing. Nccls Approved Standard M100-S9. National Committee For Clinical Laboratory Standards, Wayne.

[44]. Brand-Williams, W., Cuvelier, M.E. And Berset, C.L.W.T. Use Of A Free Radical Method To Evaluate Antioxidant Activity. Lwt-Food Science And Technology, 1995; 28; 25-30. Http://Dx.Doi.Org/10.1016/S0023-6438(95)80008-5.

[45]. Gülçin I. Antioxidant Activity Of Caffeic Acid (3,4-Dihydroxycinnamic Acid). Toxicology. 2006; 217(2-3); 213-220. 10.1016/J.Tox.2005.09.011.