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Synthesis And Biological Evaluation Of N-(4-(Morpholine-4-Carbonyl) Thiazol-2-Yl) Amino) Thiazol-4(5h)-One And Their Derivatives

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Abstract
Synthesis and biological evaluation of (E)-5-(substituted benzylidene)-2-((4-(morpholine-4-carbonyl) thaiazol-2-yl) amino) thiazol-4(5H)-one and their derivatives (6a-j) were synthesized and their structure was established with the facility of spectroscopic techniques such as FTIR, 13C NMR, 1H NMR, Mass, etc. In-vitro antibacterial, antifungal, and antioxidant activities of the synthesized compounds 6a-j have been evaluated by using ampicillin, Nystatin, and Ascorbic acid as the reference standards. Compounds 6d, 6f, 6h and 6i have revealed comparable antibacterial activity by displaying similar MIC values as the reference standard used. Although 6i bearing strongly electron withdrawing substituent's showed prominent antioxidant activities compared with remaining derivatives compared to the reference standard. The compound 6i exerts multi-target inhibitory action against different microbial.

Key Word: *Thiazole, Thiazolidinone; Antioxidant; Antimicrobial.*

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I. Introduction

Nitrogen heterocyclic compounds containing Thiazole, Thiazolidinone have been significant interest in the field of medicinal chemistry research for the scientists due to their varied pharmacological activities. [1 & 2]. The discovery of antibiotics was a turning point in human history for the treatment of fungal and bacterial infections. The drug resistance is a major global concern occurred due to early and excess use of antimicrobial drugs. There is urgent need to work for stop the drug-resistant pathogens by developing new antimicrobial drugs. WHO develops action plan to control and cure the antimicrobial resistance globally. [3-5] Current drug discovery revolves around chemical modifications of the existing drugs to create and improved efficacy against same strains of pathogen. The present threat for drug resistance and development of new strains for pathogen are creating scare in the society. [6]

The human life and economy have been impacted by past and present situations, which encourage us to develop new treatments for diseases that are likely to occur in the future due to new strains of infections that are resistant to the existing drugs. Due to untimely outbreaks of new diseases, drug resistance, lengthy approval processes, and high costs of existing limited drugs, new drugs requi.re global attention and stewardship. To prevent a calamity that doesn't yet exist and is years away from patients, screening novel antimicrobial drugs are an important task in medicinal chemistry.

In the current work, we have synthesized a hybrid of bioactive compounds which consist of Morpholine, Thiazole, and Thiazolidinone to get enhanced anti-microbial and antioxidant activity. Nitrogen heterocycles attains important role in current drug design as most of drugs involves it. Its combination with sulfur and oxygen is key in any drugs. Thiazole is a heterocycle involving nitrogen and sulfur and it belongs to azole family of compounds. Thiazole nuclei are widely distributed in nature and it is vital part of many drugs. The thaizole nuclei are having planar structure with high aromaticity. [7] Thiazole is a potential scaffold, its union with other nuclei was extensively studied for the search for new drugs with improved therapeutic activity. [8-10]

Morpholine is a scaffold with great interest in medicinal chemistry, involving in many drugs, and clinical candidates. The Morpholine ring involves electronegative oxygen which involves in decreasing the pKa and provides balanced lipophilicity, mitigating the risk for extensive plasma protein and hERG channel binding. It prevents the commencement of protein synthesis at later stages, and therefore its bacterial resistance as of date is still low. The substituted Morpholine possess a wide range of biological activities like analgesic, anti-inflammatory, antioxidant, anti-obesity, antimicrobial, and anticancer etc. [11 & 12]

Thiazolidines and its derivatives are one of the key scaffold due to their varied bioactivity, as it resemblances to thiazole. It is a five membered ring bearing nitrogen and sulfur present at first and third position to teach other. Thiazolidines nuclei by union of other nuclei showed varied biological properties, as it is part of marketed drugs like Ciglitazone, Pioglitazone, Englitazone, Ralitoline, Etozoline, and Lobeglitazone etc. mentioned in figure 1. Thiazolidines nuclei shows interactions like hydrogen bonding, ion-dipole interactions, π-π interactions with mild vander walls forces via hydrophobic bonds with various enzymes makes them show varied biological activities. Its association with different nuclei showed properties like anticancer, antimicrobial, antitumor, anti-diabetic, anti-parasitic, anti-inflammatory, anti-tubercular, antifungal, antiviral, anti-HIV etc. [13, 14] Thiazolidines involves in various reactions like multi component reactions, substitutions reaction, Nano catalysts reactions, click reactions, green chemistry reactions etc. The substitution reaction is easily possible at 2, 4, & 5 position of ring, as these are active positions in the nuclei. Thiazolidines nuclei and their derivatives showed anticancer, [15] antimicrobial, [16] anti-diabetic, [17] anti-inflammatory, [18] antiviral, [19] anti-convulsant, [20] and anti-HIV activity [21] etc. [22 &23]

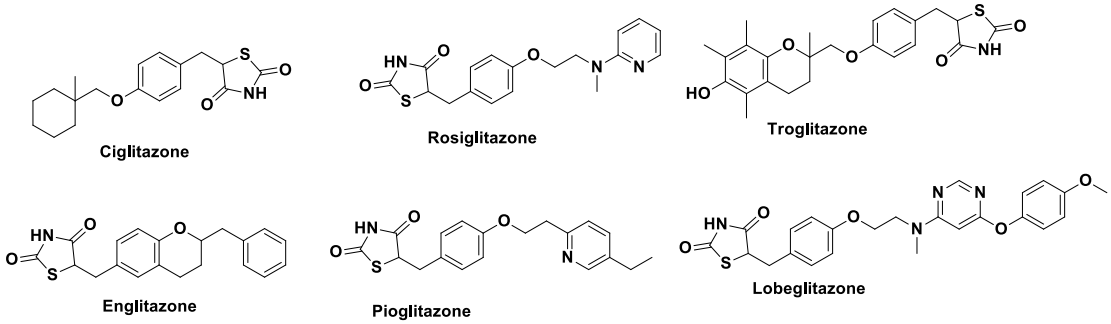
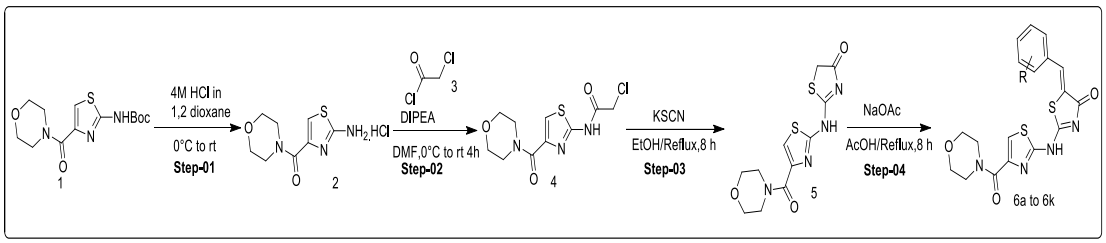


Fig. 1: : Marketed drugs with Thiazolidinone moiety.

By considering the fascinating medicinal activity of Thaizole, Morpholine and Thiazolidines we tried to incorporate them in one nuclei via amide coupling to check their antimicrobial and antioxidant activity we report here on the synthesis of some novel (E)-5-(substituted benzylidene)-2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino)thiazol-4(5H)-one (6a-6j) derivatives with expectation to obtained novel leads with increased bioactivity due to combined synergetic effects of these rings.

II. Material And Methods

All chemicals, unless otherwise specified, ware purchased from commercial sources and ware used without further purification. The major chemicals purchased from Sigma Aldrich and Avra labs. The progress of reaction was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F254 aluminum sheets, visualized by UV light. All reactions carried out under inert atmosphere. Melting points recorded on Casia-Siamia (VMP-AM) melting point apparatus and all are uncorrected. The purity of intermediates ware assessed by TLC, NMR, and HRMS. The purity of final compounds checked by NMR, HRMS, and HPLC and all structures are consistent with proposed structures characterization. The 1H NMR spectra were recorded on a 400 MHz Varian NMR spectrometer. The 13C recorded on a 100 MHz Varian NMR spectrometer. The chemical shifts ware reported as NMR spectra δppm units. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplate (m) and broad (br). Mass spectra were taken with Micro mass-QUATTRO-II of produced by WATERS Corporation.



Step-i: Synthesis of (2-aminothiazol-4-yl) (morpholino) methanone hydrochloride (2):

To the stirred solution of 4M HCl in 1, 4 Dioxane (80 mL) in tert-butyl (4-morpholine-4-carbonyl) (thiazol-2-yl) carbamate (1, 10 g, 1 equi.) was added at 0 °C. The reaction mixture was stirred at 25-30 °C for 12h. The reaction progress was monitored by TLC [10 % MeOH in DCM %, UV, Iodine, and Ninhydrin], after the complete consumption of starting material, the reaction mixture was concentrated under a vacuum to afford

off white solid. The obtained crude material was triturated with hexane (100 mL X 2) and again dried under a high vacuum to afford (2-aminothiazol-4-yl) (morpholino)methanone hydrochloride (7g, 87%) as an off-white solid. mp=173-175°C, HRMS (ESI)m/z calcd. For C8H11N3O2S: [M+H] + 213.26, observed mass 213.5.

Step-ii: Synthesis of 2-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) acetamide (4):

To the clear solution of compound 2 (7.00 g, 1 equi.) in DCM (100 mL), DIPEA (10.85 g, 3 equi.) was added at 0-5°C, followed by the addition of Chloroacetyl chloride (6.33g, 2equi.) at 0-5°C. The reaction was maintained at 25-30°C and monitored by TLC. After 4h the reaction was poured into water and washed with sodium bicarbonate solution (50 mL), brine solution (30 mL), and water (30 mL) successively. Then the organic layer was dried over sodium Sulphate, and concentrated under a vacuum to get 6 g of crude material. The obtained crude material was purified by column chromatography silica 100-200 mesh using 8-10% methanol in DCM as eluent to afford **2**-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) acetamide (5.6 g, 69%) as an off white solid. mp=169-170°C, HRMS (ESI) m/z calcd. for C10H12ClN3O3S: [M + H] + 290.73, found 291.0.

Step-iii: Synthesis of ethyl 2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino) thiazol-4(5H)-one (5):

To the stirred solution of compound 4 (5.5 g, 1.0 equi.) in Ethanol (80.0ml) was added KSCN (2.76 g, 1.5 equi.) and the reaction mixture was reflux for 8h. The reaction progress was monitored by TLC. After completion, the reaction mixtures were concentrated under vacuum for half volume to obtained solid precipitation, filter it and washed it with water (30 mL) and ethanol (30 mL) in the ration of 1:1 to afford 3.9 g of light brown solid. The obtained crude material was purified by triturated with hexane (25 mL x 2) and dried under vacuum to afford ethyl 2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino) thiazol-4(5H)-one (3.4 g, 75%) as an off white solid. mp=169-170°C, HRMS (ESI) m/z calcd. for C10H12ClN3O3S: [M + H] + 313.04, found 313.03.

Step-iv: General procedure for synthesis of (Z)-5-benzylidene-2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino) thiazol-4(5H)-one (6a-j):

To the stirred solution of 5 (0.3g,1 equi.) in ACOH (5 mL) was added NaOAc (0.47g, 6 equi.) and added substituted aldehyde (0.2g, 2 equi.) and the reaction mixture was reflux for 8h. The reaction progress was monitored by TLC. After completion, the reaction mixture was poured on crushed ice to get precipitation, filter the materiel and dry it under vacuum to get 0.4 g of crude materiel. The obtained crude was purified by trituration with hexane (20 mL), followed by 1: 1 ratio of hexane and ethyl acetate (20 mL) and finally by EtOH (3 mL x 2) filtered and dried under vacuum to afforded (6a-6j).

Analytical data of (E)-5-(substituted benzylidene)-2-((4-(morpholine-4-carbonyl) thaiazol-2-yl) amino) thiazol-4(5H)-one (6a-6j):

(E)-5-(benzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6a): Light brown solid, (81.45% yield); mp = 248°C-249°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.81 (bs, 1H), 7.98 (s, 1H), 7.77 (s, 1H), 7.68 – 7.67 (m, 2H), 7.54 – 7.53 (m, 3H), 3.97 (s, 2H), 3.72 (s, 4H), 3.66 (s, 2H); 13C NMR (500 MHz, DMSO-D6: δ 168.01, 176.21, 161.96, 156.74, 147.11, 133.86, 132.78, 130.93, 130.54, 129.66, 124.83, 123.72, 67.19, 66.72, 47.61, 43.21. HRMS (ESI) m/z calcd. For C18H16N4O3S2: [M + H] + 400.47, found 400.41.

(E)-5-(4-methoxybenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6b): Off white solid, (79.33% yield); mp = 293°C-294°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.71 (s, 1H), 7.96 (s, 1H), 7.73 (s, 1H), 7.64 – 7.63 (d, J = 6.2 Hz, 2H), 7.11 – 7.09 (d, J = 6.2 Hz, 2H), 3.96 (bs, 2H), 3.84 (bs, 3H), 3.72 (bs, 4H), 3.66 (bs, 2H); 13C NMR (500 MHz, DMSO-D6: δ 168.13, 167.38, 162.02, 161.43, 156.94, 147.07, 132.89, 132.62, 126.33, 123.41, 121.66, 115.25, 67.19, 66.73, 55.98, 47.61, 43.21; HRMS (ESI) m/z calcd. for C19H18N4O4S2: [M + H] + 430.50, found 430.54.

(E)-5-(4-chlorobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6c): Light brown solid, (77.13% yield); mp = 264°C-265°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.85 (s, 1H), 7.98 (s, 1H), 7.76 (s, 1H), 7.69 – 7.68 (d, J = 8.4 Hz, 2H), 7.61 – 7.59 (d, J = 8.4 Hz, 2H), 3.94 (bs, 2H), 3.73 – 3.69 (bs, 7H); 13C NMR (500 MHz, DMSO-D6: δ 167.92, 167.16, 161.92, 156.36, 147.13, 135.38, 132.79, 131.41, 129.74, 125.6, 123.73, 67.15, 66.70, 47.61, 43.20; HRMS (ESI) m/z calcd. for C18H15ClN4O3S2: [M + H]+ 434.92, found 434.82.

(E)-5-(4-N-dimethylaminobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6d): Yellow solid, (80.31% yield); mp = 254°C-255°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.55 (s, 1H), 7.93 (s, 1H), 7.64 (s, 1H), 7.52 – 7.50 (d, J = 8.0 Hz, 2H), 6.82 – 6.81 (d, J = 8.0 Hz, 2H), 3.99 (bs, 2H), 3.75 – 3.74 (bs, 4H), 3.67 (bs, 2H), 2.51 – 2.50 (bs, 7H); 13C NMR (500 MHz, DMSO-D6: δ 190.48, 168.36, 167.54, 162.11, 157.35, 151.90, 147.02, 134.05, 132.75, 122.98, 120.62,

117.02, 112.34, 111.54, 67.30, 66.82, 47.68, 43.19; HRMS (ESI) m/z calcd. for C20H21N5O3S2: [M + H] + 443.54, found 443.33.

(E)-5-(2-chlorobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6e): Light brown solid, (79.50% yield); mp = 259°C-260°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.93 (s, 1H), 7.99 (s, 1H), 7.87 (s, 1H), 7.71 – 7.66 (m, 2H), 7.56 – 7.49 (m, 2H), 3.89 (bs, 2H), 3.68 – 3.62 (m, 7H); 13C NMR (500 MHz, DMSO-D6: δ 167.92, 167.16, 161.92, 156.36, 147.13, 135.38, 132.79, 131.41, 129.74, 125.6, 123.73, 67.15, 66.70, 47.61, 43.20; HRMS (ESI) m/z calcd. for C18H15ClN4O3S2: [M + H] + 434.92, found 434.89.

(E)-5-(3, 5-dichlorobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6f): Light brown solid, (74.43% yield); mp = 261°C-262°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.95 (s, 1H), 7.98 (s, 1H), 7.87 – 7.86 (d, J = 6.4 Hz, 1H), 7.79 (s, 1H), 7.70 – 7.68 (d, J = 6.8 Hz, 1H), 7.75 – 7.60 (d, J = 7.6 Hz, 1H), 3.87 (bs, 2H), 3.69 – 3.33 (bs, 7H); 13C NMR (500 MHz, DMSO-D6: δ 167.78, 166.77, 161.85, 156.07, 147.08, 135.87, 135.70, 131.01, 130.81, 130.45, 129.23, 128.48, 126.76, 126.89, 67.04, 66.64, 47.56, 43.19; HRMS (ESI) m/z calcd. for C18H14Cl2N4O3S2: [M + H] + 469.36, found 469.29.

(E)-5-(2-furoyl)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6g): Off white solid, (75.25% yield); mp = 255°C-256°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.67 (s, 1H), 7.96 – 7.94 (d, J = 6.4 Hz, 2H), 7.58 (s, 1H), 7.14 (s, 1H), 6.80 (s, 1H), 3.96 (s, 2H), 3.76 (bs, 4H), 3.67 (s, 2H); 13C NMR (500 MHz, DMSO-D6: δ 168.23, 167.17, 162.07, 158.0, 150.16, 147.57, 147.03, 123.15, 121.57, 119.41, 119.10, 114.31, 67.21, 66.76, 47.76, 43.26; HRMS (ESI) m/z calcd. for C16H14N4O3S2: [M + H] + 390.44, found 390.52.

(E)-5-(5-boromo-3-flourobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6h): Brown solid, (78.35% yield); mp = 281°C-282°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.98 (s, 1H), 8.0 (s, 1H), 7.89 – 7.88 (d, J = 6.4 Hz, 1H), 7.74 (s, 1H), 7.50 – 7.49 (d, J = 6.8 Hz, 1H), 7.38 – 7.35 (d, J = 7.6 Hz, 1H), 3.89 (bs, 2H), 3.65 – 3.59 (bs, 8H); 13C NMR (500 MHz, DMSO-D6: δ 167.82, 166.82, 161.85, 147.04, 135.89, 135.58, 129.44, 124.09, 120.10, 119.50, 119.32, 116.54, 116.35, 66.91, 66.67, 47.53, 43.19; HRMS (ESI) m/z calcd. for C18H14BrFN4O3S2: [M + H] + 497.36, found 497.29.

(E)-5-(4-chloro-2-flourobenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6i): Light brown solid, (80.43% yield); mp = 264°C-265°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.77 (s, 1H), 8.45 – 7.01 (m, 11H), 4.40 – 3.01 (m, 14H); 13C NMR (500 MHz, DMSO-D6: δ 167.83, 166.95, 161.88, 161.69, 159.66, 156.09, 147.11, 136.49, 130.74, 128.39, 126.11, 125.91, 123.85, 122.72, 121.02, 117.64, 117.44, 67.07, 66.65, 47.57, 43.15; HRMS (ESI) m/z calcd. for C18H14ClFN4O3S2: [M + H] + 452.91, found 452.86.

(E)-5-(4-chloro-2-hydroxybenzylidene)-2-((4-(morpholine-4-carbonyl)thaiazol-2-yl)amino)thiazol-4(5H)-one (6j): Off white solid, (79.12% yield); mp = 219°C-220°C; IR: νmax/cm −1 = 2857, 1693, 1594, 1365, 1166; 1H NMR (500 MHz, DMSO-D6): δ 12.80 (s, 1H), 10.96 (bs, 1H), 10.85 (s, 1H), 10.13 (s, 1H), 7.97 (s, 1H), 7.89 (s, 1H), 7.56 (d, J = 6.4 Hz, 1H), 7.54 – 7.44 (m, 3H), 6.96 – 6.90 (m, 2H), 3.97 (bs, 2H), 3.65 (bs, 7H), 3.34 (s, 9H);13C NMR (500 MHz, DMSO-D6: δ 167.92, 167.16, 161.92, 156.36, 147.13, 135.38, 132.79, 131.41, 129.74, 129.6, 123.73, 67.15, 66.70, 47.61, 43.20; HRMS (ESI) m/z calcd. for C18H15ClN4O4S2: [M + H]+ 450.92, found 450.82.

III. Result

Biological Activity

Antimicrobial activity

All the synthesized compounds were investigated for the in vitro antimicrobial studies. Novel heterocyclic hybrid derivatives (6a-j) consisting scaffold such as Morpholine, Piperazine, Thiazole were evaluated for antibacterial and antifungal activity by using the micro broth dilution method against a set of Gram-positive (Bacillus subtilis NCIM 2063 and Staphylococcus aureus NCIM 2079), gram-negative (Escherichia coli NCIM 2065; PV: Proteus vulgaris NCIM 2813) bacterial microorganisms and fungal microorganisms such as Aspergillus Niger NCIM 501, Candida albicans NCIM 3471 by using ampicillin and Nystatin as a reference standard respectively.

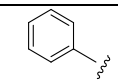
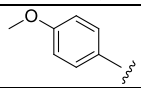
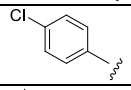
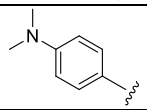
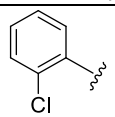
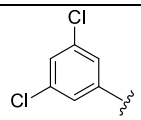
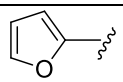
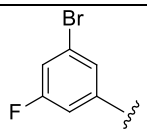
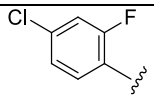
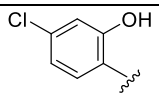
Compound	R =	Gram +ve Bacteria		Gram - ve Bacteria		Fungal Microorganism	
		BS	***SC***	***EC***	***PV***	***AN***	***CA***
6 a		500	250	250	375	**250**	250
6 b		125	375	187.5	187.5	500	**125**
6 c		250	375	187.5	375	**187.5**	375
6 d		187.5	250	250	**250**	375	125
6 e		250	375	250	125	**187.5**	**187.5**
6 f		**125**	375	187.5	**250**	375	500
6 g		500	375	187.5	375	**187.5**	375
6 h		187.5	375	187.5	**187.5**	**187.5**	**187.5**
6 i		187.5	**187.5**	187.5	375	**250**	375
6 j		375	375	250	375	**250**	250
Standard	Ampicillin	125	187.5	125	250	NA	NA
	Nystatin	NA	NA	NA	NA	250	187.5

Table 1: Antibacterial and antifungal activities of compound (6a-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 Positive and negative bacterial microorganisms); Nystatin (for fungal microorganisms), NA = Not applicable.

In-vitro Antibacterial activity:
It is perceived that, In fig. 2

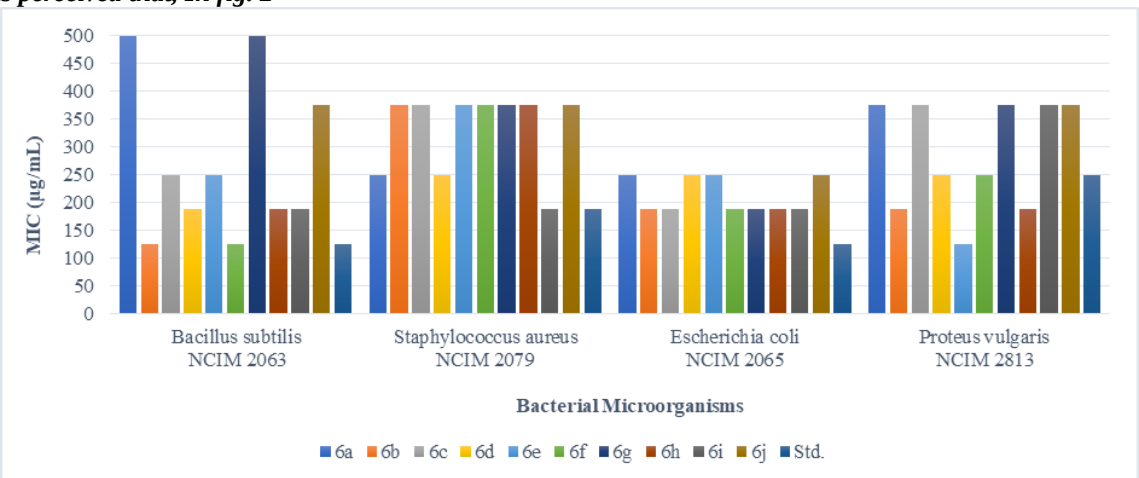
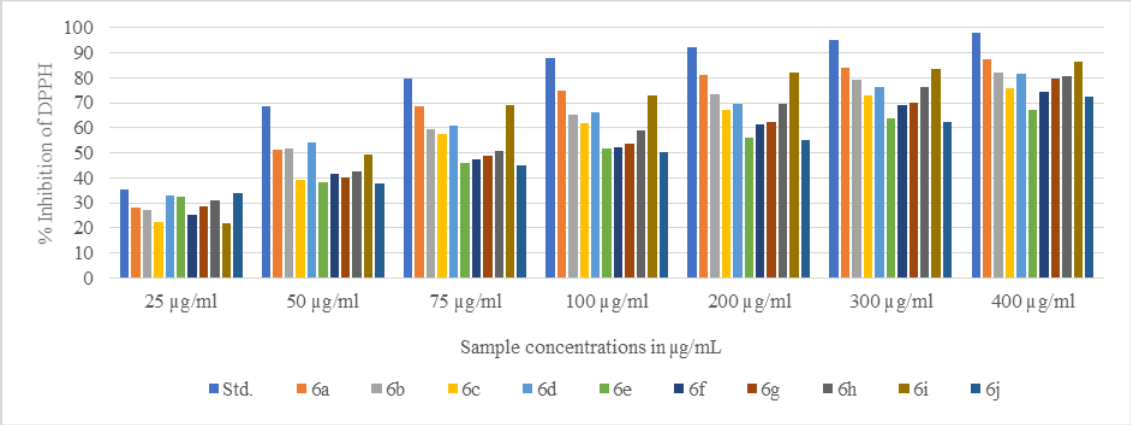


Figure 2: Effect of hybrid compounds on the gram +ive and gram -ive bacterial inhibition.

Conc. µg/ml	DPPH % Inhibition										
		6 a	**6 b**	**6 c**	**6 d**	**6 e**	**6 f**	**6 g**	**6 h**	**6 i**	**6 j**
25	35.39	28.31	27.07	22.47	**33.07**	32.58	25.01	28.65	31.06	22.03	**34.02**
50	68.64	51.28	51.79	39.43	53.93	38.20	41.55	40.12	42.56	49.52	37.56
75	79.89	**68.65**	59.53	57.55	60.67	46.06	47.56	48.96	51.02	**69.21**	45.20
100	87.89	**74.78**	65.03	62.04	66.29	51.58	52.36	53.64	59.14	**73.20**	50.22
200	92.13	**81.02**	73.52	67.41	69.66	56.17	61.23	62.54	69.51	**82.01**	55.26
300	95.13	**84.27**	79.02	73.03	76.40	64.04	69.21	70.12	76.51	**83.56**	62.30
400	98.26	**87.27**	82.02	76.03	**81.40**	67.04	74.25	79.55	**80.55**	**86.62**	72.45

Table 2: Antioxidant activities of compound (6a-j) (Values are mean ± SEM of triplicate determinations)



The antioxidant activity data reveals the derivatives 6a, 6d, 6h, 6i and 6j have displayed moderate to good activity against DPPH free radical. Among studied hybrid compounds in this study, compound 6a and 6i have displayed maximum DPPH % of inhibition and comparable to the activity of the ascorbic acid used as a standard. Presences of Chlorine and fluorine substituent at Meta position of phenyl ring have shown 86.62 % of inhibition of free radical against standard with 98.26 % inhibition.

IV. Discussion

In-vitro Antifungal activity

In-vitro antifungal activity of (6a-j) has been determined and displayed in table-1. It was summarized that given series of hybrid compounds have been shown good growth inhibition of selected fungal pathogens Aspergillus Niger NCIM 501 and Candida albicans NCIM 3471 pathogens. Except Compounds 6d and 6f have displayed almost similar activity as a Nystatin used as a reference standard used.

In-Vitro Antioxidant activity

The hybrid series prepared from Thiazole, Thiazolidinone and Morpholine 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. It was clearly seen from the results shown in table 2 that the radical scavenging activity of the compounds found to be dependent in a concentration manner.

In-Vitro antibacterial activity

According to the results obtained and shown in Table 1, it is observed that all compounds have the ability to suppress the growth of different strains of bacteria to varying degrees. It was noticed that most of the compounds in the series have shown better inhibition of gram-negative Proteus vulgaris NCIM 2813 bacterial microorganisms. Moreover, compounds 6f have shown potent and broad-spectrum activity against all tested microorganisms.

Compounds 6d, 6f, 6h and 6i have revealed comparable antibacterial activity by displaying similar MIC values as the reference standard used.

DPPH Assay method for the evaluation of in-vitro Antioxidant Activity

The antioxidant activity data reveals the derivatives 6a, 6d, 6h, 6i and 6j have displayed moderate to good activity against DPPH free radical. Among studied hybrid compounds in this study, compound 6a and 6i have displayed maximum DPPH % of inhibition and comparable to the activity of the ascorbic acid used as a

standard. Presences of Chlorine and fluorine substituent at Meta position of phenyl ring have shown 86.62 % of inhibition of free radical against standard with 98.26 % inhibition.

Materials and methods

The antibacterial and antifungal activities were done by using Agar well diffusion method at 500µg/ml concentration and studied against Amoxicillin and Fluconazole as a standard. Antioxidant activity was evaluated with the help of the DPPH method against the Ascorbic acid standard. Bacterial and antifungal cultures were obtained from Merck.

In-vitro Antibacterial and antifungal activity by Agar well diffusion method:

Agar Well Diffusion Method was used to determine the antibacterial and antifungal activity of the test substance. 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 were each cultured individually for 24 hours. We created sterile Nutrient agar plates for bacterial cultures and sterile Chloramphenicol Yeast Glucose Agar plates for fungus cultures. A 0.2 ml culture of each type of microorganism was dispersed on various plates using sterile swabs. Four or five wells in the agar were created using an 8.0 mm cork borer on each plate. As a stock solution, a 10 mg/ml suspension of the test substance was produced in Dimethyl Sulfoxide (DMSO). Each well received 50µg/ml of the stock solution. [26-28]

Procedure: A volume of 100 µL of synthesized compound (Series 1-S1-S10) 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). 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 case of Fungi. The plates were prepared in triplicates and placed in an incubator set at 37 °C for 18-24 h. for bacteria and 25 °C for 48 h. 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 spectrophotometric 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 compounds.

DPPH Assay method for the evaluation of in-vitro Antioxidant Activity

DPPH assay method has been used for the determination of the in-vitro antioxidant activity of the novel series of compounds in this study. 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. [29 &30] The stability of electrons 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 (6a to 6j) and the ascorbic acid are measured at different concentrations between 25-400 µg/ml and reported.

V. Conclusion

Using simple chemistry to link two or more heterocyclic scaffolds, this study aims to synthesize and screen novel hybrid compounds against gram positive/negative bacterial and fungal microorganisms with antioxidant capabilities. Based on our study, the majority of synthesized compounds containing Thiazole, Thiazolidinone exhibited moderate to good antibacterial and antifungal properties. The derivatives 6a, 6h & 6i showed moderate to good antioxidant activity. The derivatives 6d and 6f have revealed good antifungal activity against Nystatin (Aspergillus Niger NCIM 501 and Candida Albicans NCIM 3471) representing the zone of inhibition analogous to the reference standard used. The compound having strong electron withdrawing and deactivating derivative 6i has come out as a promising compound which has shown antibacterial, antifungal and antioxidant activity as well. In spite of the fact that synthetic compounds have well-defined zones of inhibition against limited microbes, they still need to be strengthened. As a result, these compounds can be further investigated for other bacterial and fungal microorganisms with few structural modifications, may show favorable activity, and would help discover promising compounds.

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