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Synthesis and Biological Evaluation of N-(4-(Morpholine-4-Carbonyl)Thiazol-2-yl)-2-(piperazin-1-yl) Acetamide Hydrochloride and Their Sulfonamide Derivatives

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Abstract:- A series of novel heterocyclic hybrids, N-(4-(morpholine-4-carbonyl) thiazol-2-yl)-2-(piperazin-1-yl) acetamide hydrochloride and its sulfonamide derivatives were synthesized (compound 6a-j) and their structure was established with the facility 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 6a-j have been evaluated by using ampicillin, Nystatin, and Ascorbic acid as the reference standards. Compounds 6b, 6c, 6e, 6g, and 6j have revealed comparable antibacterial activity by displaying similar MIC values as the reference standard used. Although antioxidant activities are seen as ordinary compared to the reference standard, interestingly compounds 6b, 6e, and 6g have displayed multi-target inhibitory action against different microbial, making it a possible choice as a single drug for the cater of multiple ailments.

I. INTRODUCTION

The 19th Century is considered the inception of the antimicrobial era after the discovery of penicillin by Scottish scientist Alexander Fleming which was further successfully used to treat Eye infection followed by treatment against streptococcal meningitis[1] in 1942. The success of penicillin against infectious disease encouraged clinicians to evaluate naturally occurring compounds as antimicrobial agents. The Discovery of Sulpha drugs is crucial in reducing the mortality rate of acute meningococcal meningitis from 70-90% to 10% which further boosts the discovery and use of its life-threatening infectious disease.[2] Many new and wide range of antimicrobial agents in life-threatening infectious diseases. Many new and wide ranges of antimicrobial agents like aminoglycopeptide, Chloramphnicol, Tetracyclin, Quinolones were discovered providing doctors with several treatment options for the treatment of most infectious diseases. Many agents were introduced by doing chemical modification of existing drugs to create an improved analog that will have higher efficacy against diseases caused by a pathogen which were resistant to the previous generation of the same class.

However, the expanded use of antimicrobials for less severe illnesses raised the concern of antimicrobial resistance where clinicians observed an increased rate of resistance among previously susceptible organisms which have in turn limited the treatment option for some serious diseases.[3-6] In recent history, microbial illnesses like SARS (Corona),[7,8] H1N1 (Swine Flu), Zika,[9] and Ebola[10,11] have caused a crisis and Pandemic. The past and present situations impacted our life and economy as well. The Spread of this resistance now demands the use of existing antimicrobial agents judiciously and the introduction of new antimicrobial agents with better bioactivity against infectious diseases.

Additionally, the process of oxidation in the human body damages cell membranes and other structures, including cellular proteins, lipids, and DNA. Oxidation can be accelerated by stress, cigarette smoking, alcohol, sunlight, pollution, and other factors.[12] We are currently afflicted with several serious illnesses including cancer, autoimmune diseases, aging, cardiovascular disease, and neurological disorders, and we are susceptible to contracting these illnesses as a result of oxidative damage to cells and tissues brought on by reactive oxidative species. Antioxidants scavenge free radicals from the body cells and prevent or reduce the damage caused by oxidation.[13] Antioxidants are responsible for better immunity against infections, autoimmune diseases, and cancer.[14-17] Hence, it is required to design potent antioxidizing agents in collaboration with antibacterial and anti-inflammatory agents.

Many Bioactive compounds in medicinal chemistry involved heterocyclic compounds containing nitrogen, oxygen, and Sulphur atoms such as Furan, Coumarin, Thiazole, Thiazolidinone, Imidazole, Pyrrole, Morpholine, Piperazine, Pyran, Quinoline, Pyridine, Pyrimidine, Isatin, Indole, have been reported and synthesized different novel derivatives.[18] In the current work, we concentrated on the fusion of individual bioactive compounds which consist of Piperazine, Morpholine, Thiazole, and Sulfonamide to get enhanced anti-microbial and antioxidant activity.

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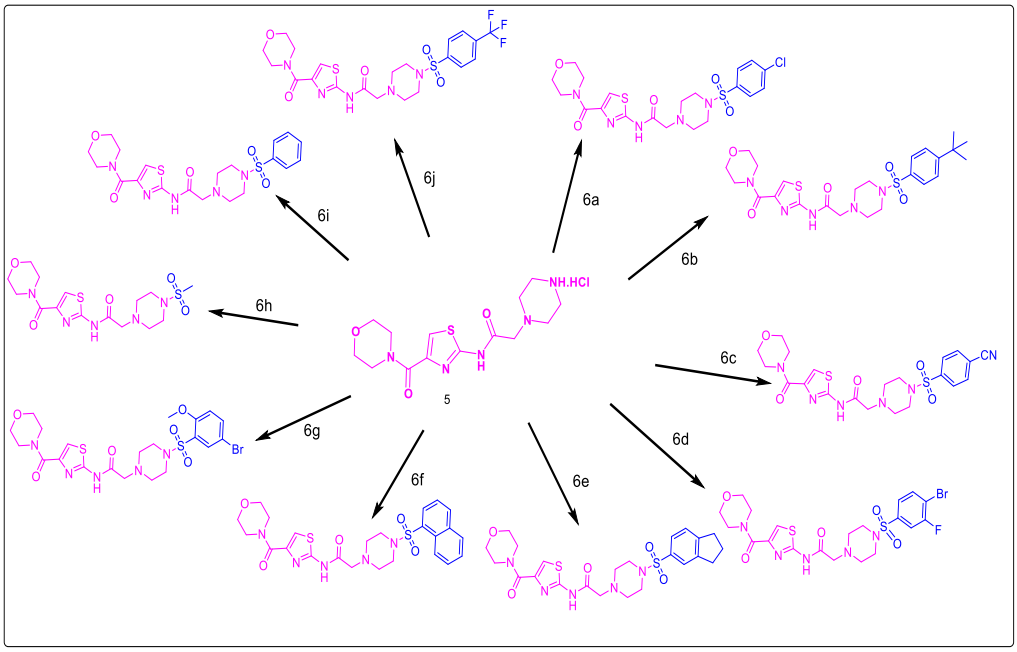

Fig. 1: Heterocyclic hybrid compound containing different pharmacophores.

Above mentioned scaffolds were selected based on their known bioactivity against multiple ailments.

Morpholine is a privileged pharmacophore with a wide range of pharmacological activities.[19,20] The active pharmacophores consist of Morpholine accountable for anticancer, anti-inflammatory, antiviral, anticonvulsant, antihyperlipidemic, antioxidant, antimicrobial, and antileishmanial activity.[19,20] It prevents the commencement of protein synthesis at later stages, and therefore its bacterial resistance as of date is still low. These findings encouraged us to select morpholine to design novel and potent morpholine derivatives as broad-spectrum antimicrobial agents.

The piperazine molecule has been classified as a privileged structural motif in drug discovery.[21,22] It is an important pharmacophore found in numerous marketed drugs such as antibiotics, anticancer, antimicrobials, antipsychotics, and antidepressants. e.g., Norfloxacin, Ciprofloxacin.[23]

Thiazole has a wide range of biological activities such as antioxidant, analgesic, antibacterial, anticancer, antiallergic, antihypertensive, anti-inflammatory, antimalarial, antifungal, and antipsychotic.[24] The presence of Thiazole in hybrid may help in enhancing bioactivity.[25,26]

Sulfonamide is selected as a hybrid as it possesses a wide range of pharmacological activities such as anti-bacterial, anti-fungal, anti-cancer, and anti-inflammatory.[27,28] Sulfa drugs effectively inhibit folic acid synthesis in the microorganism and subsequently inhibit the multiplication of microorganisms.[29,30] Sulfa derivatives are popular as they are well tolerated by patients and relatively inexpensive.[31]

II. RESULTS AND DISCUSSION

A. In-vitro Antibacterial Activity

Novel sulfonamide derivatives of hybrid scaffold consisting of Morpholine, Piperazine, Thiazole 6a-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.

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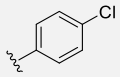
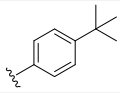
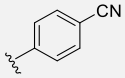
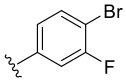
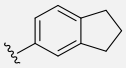
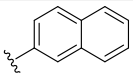
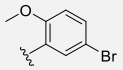
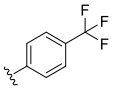
Table 1: Antibacterial and antifungal activities of compound 6a-j against *BS:* Bacillus subtilis NCIM 2063; *SA*: Staphylococcus aureus NCIM 2079, *EC*: Escherichia coli NCIM 2065; *PV*: Proteus vulgaris NCIM 2813, *AN*: Aspergillus Niger NCIM 501; and *CA*: Candida albicans NCIM 3471 microorganisms, standard: Ampicillin (For gram Positive and negative bacterial microorganisms); Nystatin (for fungal microorganisms).

Compound	Substituent R=	Minimum Inhibitory Concentration (MIC) in µg/mL					
		Gram +ve Bacteria		Gram - ve Bacteria		Fungal Microorganism	
		BS	***SA***	***EC***	***PV***	***AN***	***CA***
6a		187.5	250	187.5	500	375	250
6b		250	375	187.5	**250**	375	**187.5**
6c		187.5	250	250	**250**	500	250
6d		187.5	375	250	375	**250**	250
6e		375	250	375	**250**	**250**	**187.5**
6f		187.5	375	187.5	500	375	250
6g		375	250	375	**250**	**250**	250
6h		187.5	250	250	375	375	375
6i		375	250	187.5	375	375	375
6j		187.5	**187.5**	187.5	375	**250**	250
	Standard	**125**	**187.5**	**125**	**250**	**250**	**187.5**

According to the results obtained and shown in Table1, 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 **6b, 6c, 6e,** and **6g** have shown the same MIC values as reference standards against gram-negative bacterial microorganisms. It is observed that phenyl, Naphthalene, and methyl substitution have a neutral impact on antibacterial activity whereas indane substitutions have a positive impact on antimicrobial activity. Further, a substituted phenyl ring with the presence of an electron donating group like Tert butyl at the para position and methoxy group at the ortho position has enhanced antimicrobial activity against Proteus vulgaris NCIM 2813. Substitution of the Cyno group at para position (**6c**) and bromo group at meta position along with methoxy group (**6g**) indicated good antimicrobial activity against (Proteus vulgaris NCIM 2813). The inhibitory action of all the synthesized novel compounds against one of the chosen gram-negative bacterial microorganisms i.e. Escherichia coli was seen as ordinary as compared to a reference standard.

B. In-vitro Antifungalactivity

In-vitro antifungal activity of compound 6a-j has been determined and displayed in Table 1. It was summarized that given series of hybrid compounds have displayed the ability to inhibit the growth of Aspergillus Niger and Candida albicans to a different extent. Compound 6e displayed the same MIC value as of reference standard against both Aspergillus Niger NCIM 501 and Candida Albicans NCIM3471. Whereas compounds 6d, 6g, and 6j exhibit similar MIC values as of reference standard against Aspergillus Niger NCIM 501.

C. In-Vitro Antioxidantactivity

Novel sulfonamide derivatives of hybrid scaffold consisting of Morpholine, Piperazine, Thiazole **6a-j** were evaluated for their in vitro antioxidant activity at several concentrations ranging from 25 to 400 μg/mL of compounds and standard by DPPH assay.

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

Conc. µg/mL	DPPH % Inhibition										
	Standard	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**
25	35.39	20.14	21.56	22.14	26.24	21.02	22.48	21.07	20.63	22.36	21.48
50	68.64	42.11	45.26	40.21	41.02	39.65	38.45	40.02	39.60	42.36	41.05
75	79.89	51.26	55.42	48.32	51.78	52.36	53.12	54.11	51.22	50.33	49.16
100	87.89	58.95	61.22	56.33	61.21	60.21	63.22	64.11	61.28	60.14	59.22
200	92.13	63.14	67.55	67.89	65.23	62.58	69.33	67.45	62.20	64.11	68.52
300	95.13	69.32	72.04	75.14	69.54	65.21	71.69	70.11	64.20	68.55	73.65
400	98.26	79.55	82.65	**85.75**	79.54	75.22	81.24	80.14	74.21	78.50	**83.32**

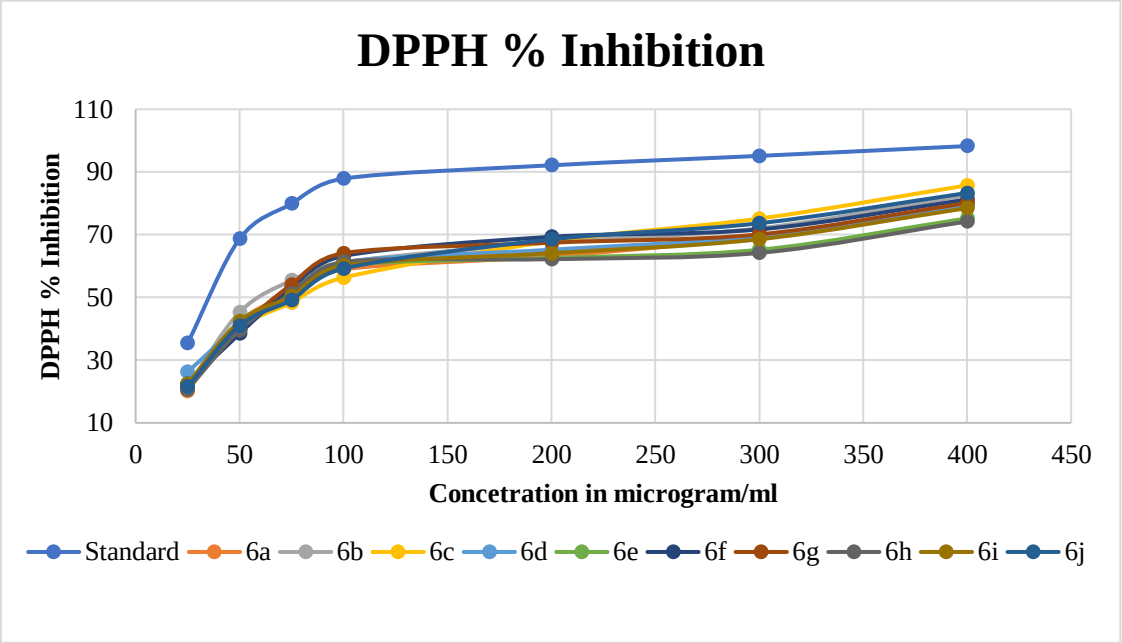

Fig. 2: Effect of Novel hybrids on inhibition of DPPH free radical

It was 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 **6c** and **6j** have displayed good DPPH % of inhibition at 400 µg/mL concentration and are comparable to the activity of the ascorbic acid used as a standard as shown in Fig. 2. The presence of Cyno substituent at the para position of the Phenyl ring and substitution of trifluoro methyl group at the para position have shown **85.75 %** and **83.32 %** of inhibition of free radicals against the standard with **98.26 %** inhibition.

III. MATERIALS AND METHODS

All chemicals such as Boc Piperazine, Chloroacetyl chloride, Sodium hydroxide, Boc-anhydride, DIPEA, TEA, and solvents such as ethanol, MDC, DMF, Ethyl acetate, n-Hexane were obtained from Sigma-Aldrich, Avra and used without purification. Melting points are taken using apparatus. Infrared spectra were recorded on a Bruker spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained from a 500 MHz spectrometer. Biological activity evaluation was done at Aster analytics research institute. The antibacterial and antifungal activities were accomplished by using the micro broth dilution method against Amoxicillin and Nystatin as a standard. Antioxidant activity was accomplished by using the DPPH method against the Ascorbic acid standard.

A. Chemistry:

Novel series of heterocyclic hybrids have been synthesized by the multi-step synthesis protocol as described in Scheme 1. Tert-butyl (4-(morpholine-4-carbonyl) thiazol-2-yl) carbamate [Compound **1**] was synthesized by condensation of Morpholine with t-Butyl-1,3-Thiazol-2-yl carbamate by using HBTU, DIPEA, 1-HOBt. (2-aminothiazol-4-yl) (morpholino) methanone hydrochloride [Compound **2**] was prepared by deprotection of Boc protection using 4 M HCl in 1,4-Dioxane followed by coupling of Chloroacetyl chloride using DIPEA as base to obtain 2-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) Acetamide [compound **3**].

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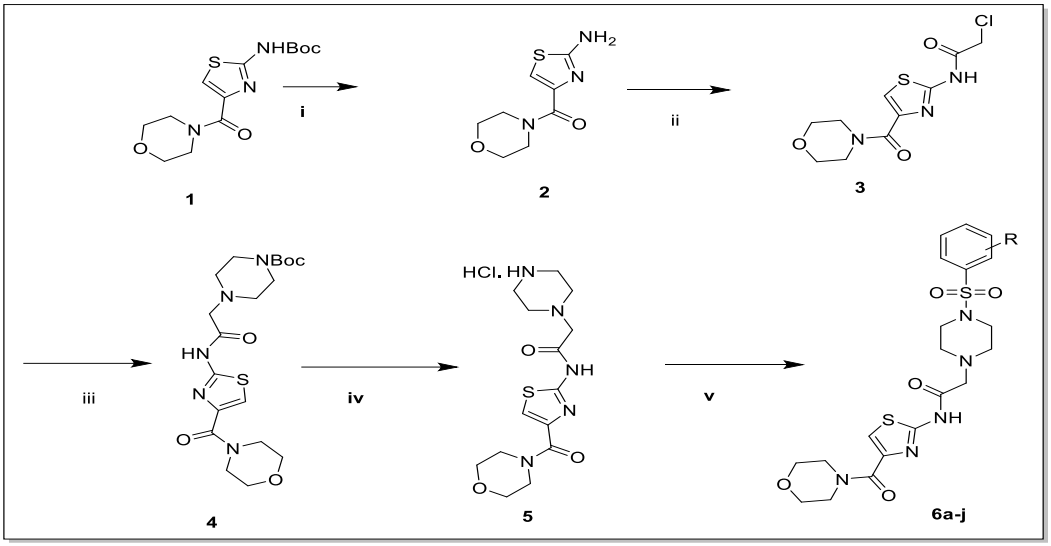

Fig. 3: Chemistry equations

Scheme-1: (i) 4M HCl in 1,4 Dioxane, 0 °C to rt,12 h, (ii) Chloroacetyl chloride, DIPEA, DMF 0 °C to rt,4 h, (iii) DIPEA, Boc Piperazine, DMF, rt,12 h, (iv) 4M HCl in 1,4 Dioxane, 0 °C to rt,12 h.(v) Respective sulfonyl chloride, DIPEA.DMF,0 °C to rt,12 h. Compound **3** was further allowed to react with Boc-Piperazine to form Tert-butyl 4-(2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino)-2-oxoethyl) piperazine-1-carboxylate [compound **4**], which is then exposed to highly acidic condition to afford N-(4-(morpholine-4-carbonyl) thiazol-2-yl)-2-(piperazin-1-yl) Acetamide hydrochloride [Compound **5**] by removal of Boc protecting group. Compound **5** was then finally used to synthesize series by reacting with different Sulphonyl chlorides to yield compound **6a-j.**

IV. CHEMICAL PROCEDURE

- **Preparation of (2-aminothiazol-4-yl) (morpholino)methanone hydrochloride (Compound 2):** To the stirred solution of 4M HCl in 1, 4 Dioxane (80 mL), compound 1 (10 g, 0.031M) was added at 0 °C. The reaction mixture was stirred at 25-30 °C for 12 h. The reaction is monitored by TLC [10 % MeOH in DCM %, UV, Iodine, and Ninhydrine], after the complete conversion 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 a 7 g final product as an off-white solid. (87% yield); mp=173-175 °C, HRMS (ESI)m/z calcd. For $C_8H_{11}N_3O_2S$: $[M+H]^+$ 213.26, observed mass 213.5.
- **Synthesis of 2-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) Acetamide (Compound 3):** To the clear solution of compound 2 (7.00 g, 0.028M) in DCM (100 mL), DIPEA (10.85 g, 3 equivalents.) was added at 0-5 °C, followed by the addition of chloroacetyl chloride (6.33g, 2 equivalent) at 0-5 °C. The reaction was maintained at 25-30 °C and monitored by TLC. After 4 h the reaction was poured into water and washed with sodium bicarbonate solution, brine solution, and water 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 5.6 g of **2**-chloro-N-(4-(morpholine-4-carbonyl) thiazol-2-yl) Acetamide (Compound 3) as an off white solid (68.94% yield). mp=169-170 °CHRMS (ESI) m/z calcd. for $C_{10}H_{12}ClN_3O_3S$: $[M + H]^+$ 290.73, found 291.0
- **Preparation of tert-butyl 4-(2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino)-2-oxoethyl) piperazine-1-carboxylate (compound 4):** To the stirred solution of Boc-Piperazine (3.85 g, 1.2 equivalent) in DMF, DIPEA (6.67 g, 3 equivalent) was added at 0-5 °C followed by the addition of compound 3 (5 g,0.0172 mol.) at 0-5 °C. The reaction mixture was stirred at RT and monitored by TLC. After 16 h, the reaction mixture was poured on crushed ice and then extracted with ethyl acetate, and the organic layer was washed with water and brine. The resulting organic layer was dried over sodium sulfate and concentrated under a vacuum to afford 7 g crude material. The crude product was purified by column chromatography silica 100 to 200 mesh using 5 to 6 % methanol in DCM as an eluent to afford 5 g of tert-butyl 4-(2-((4-(morpholine-4-carbonyl) thiazol-2-yl) amino)-2-oxoethyl) piperazine-1-carboxylate (compound 4) as an off white solid (65.92% Yield). mp= 146- 148 °C HRMS (ESI) m/z calcd. for $C_{19}H_{29}N_5O_5S$: $[M + H]^+$ 440.19, found 440.6.
- **Synthesis of N-(4-(morpholine-4-carbonyl) thiazol-2-yl)-2-(piperazin-1-yl) Acetamide hydrochloride (Compound 5):** To the stirred solution of 4M HCl in 1, 4 Dioxane (50 mL), compound 4 (5 g, 1 equivalent) was added at 0-5 °C. The reaction mixture was stirred at 25-30 °C and it was monitored by TLC. After 12 h the reaction mixture was concentrated under a vacuum to afford 5 g semisolid. The obtained semisolid was triturated with hexane and ethyl acetate [1:1] (50 mL X 2) and again dried under a high vacuum to afford 4 g N-(4-(morpholine-4-carbonyl) thiazol-2-yl)-2-(piperazin-1-yl) Acetamide hydrochloride (Compound 5) as a white solid (93.54% yield) mp=160-162 °C. HRMS (ESI) m/z calcd. for $C_{33}H_{50}N_{10}O_8S_2$: $[M + H]^+$ 779.95, found 780.

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- **General procedure for Preparation of (Compound 6 a-j):** To the stirred solution of compound 5 (1equivalent) in DMF, DIPEA (3 equivalent) was added at 0-5 °C, followed by the addition of different derivatives of sulfonyl chloride (1.2 equivalent). The reaction mixture was stirred at 25-30 °C till the complete conversion of compound 5 by TLC. After complete consumption of compound 5, the reaction mixture was poured on chilled water and extracted with ethyl acetate to afford crude material which was purified by column chromatography silica 100 to 200 mesh using 50% to 80 % ethyl acetate in hexane as an eluent to afford final products [**Compound 6a-j]** as off white to light brown solid.
- **Compound 6 a**: Light brown solid, (79.33% yield); mp = 178°C-179 °C; IR: νmax/cm $^{-1}$ = 2857, 1693, 1594, 1365, 1166; ^{1}H NMR (500 MHz, DMSO): δ 3.45(8H, *Broad*), 3.61 (8H, *Broad*), 4.14(2H, Broad), 7.37-7.60 (4H, 7.37 (d, *J* = 5Hz), 7.59 (d, *J* = 5 Hz)), 7.70 (1H, s); ^{13}C NMR (500 MHz, DMSO: δ 51.73 (7C, s), 66.73 (2C, s), 119.0 (1C, s), 127.93 (2C, s), 128.13 (2C, s), 130.15 (1C, s), 130.26 (1C, s), 139.26 (1C, s), 144.83 (1C, s), 147.82 (1C, s), 162.80(1C, s).; HRMS (ESI) m/z calcd. for $C_{20}H_{24}ClN_5O_5S_2$: [M + H]$^+$ 515.02 Da, found 514.3Da.
- **Compound 6 b:** off white solid, (80.69% yield); mp = 149-150 °C; IR: νmax/cm $^{-1}$= 2963, 1694, 1648, 1595, 1396, 1167; ^{1}H NMR (500 MHz, DMSO):δ 1.27(9H, *Singlet*), 3.41-3.47 (10H, *Broad*), 3.56-3.61(8H, Broad), 7.32-7.52 (4H, 7.32-7.34 (d, *J* = 10Hz), 7.52-7.54 (d, *J* = 10 Hz)), 7.70 (1H, s); ^{13}C NMR (500 MHz, DMSO: δ 31.57 (3C, s), 39.49 (1C, s), 51.82 (7C, s), 66.24 (2C, s), 118.95 (1C, s), 125.74 (1C, s), 126.91(1C, s), 126.98 (1C, s), 128.19 (1C, s), 128.23 (1C, s), 131.63 (1C, s), 144.83 (1C, s), 146.19 (1C, s), 151.24 (1C, s), 162.80(1C, s); HRMS (ESI) m/z calcd. for C24H33N5O5S2: [M + H]$^+$ 536.68, found 536.3
- **Compound 6 c:** Light brown solid, (78.21% yield); mp = 121-122 °C; IR: νmax/cm $^{-1}$ = 2853, 2232, 1686, 1620, 1524, 1164; ^{1}H NMR (500 MHz, DMSO): δ 2.51-2.61(4H, *Broad*), 2.95-3.00 (4H, *Broad*), 3.32 (2H, Singlet), 3.38-3.61(8H, Broad), 7.60 (1H, s), 7.92-8.16 (4H, 7.92-7.44 (d, *J* = 10Hz), 8.15-8.16 (d, *J* = 5Hz; ^{13}C NMR (500 MHz, DMSO: δ 52.6 (6C, s), 59.75 (1C, s), 66.69 (2C, s), 116.18 (2C, s), 118.12(1C, s), 127.51 (1C, s), 128.76(1C, s), 132.77 (1C, s), 134.07 (1C, s), 139.77(1C, s), 144.61 (1C, s), 157.34(1C, s), 163.08 (1C, s), 169.21 (1C, s); HRMS (ESI) m/z calcd. For C21H24N6O5S2 : [M + H]$^+$ 505.3, found 505.3
- **Compound 6 d:** Light brown solid, (81.50% yield); mp = 116-117 °C; IR: νmax/cm $^{-1}$ = 2921, 2853, 1692, 1233, 1163; ^{1}H NMR (500 MHz, DMSO): δ 2.60-3.00(10H, *Broad*), 3.60(8H, Broad), 7.52-7.54(1H, d, *J* = 10Hz), 7.60 (1H, s), 7.73-8.06 (2H, 7.73-7.76 (d, *J* = 15Hz), 8.03-8.06 (d, *J* = 15Hz)); ^{13}C NMR (500 MHz, DMSO) : δ 46.25 (2C, s), 51.75 (2C, s), 59.76 (2C, s), 60.22 (1C, s), 66.78 (2C, s), 114.70 (1C, s), 116.23 (1C, s), 118.12 (1C, s), 125.5 (1C, s), 135.40 (1C, s), 136.93(1C, s), 144.60 (1C, s), 157.35 (1C, s), 157.76 (1C, s), 163.08 (1C, s),169.24 (1C, s).; HRMS (ESI) m/z calcd. for C20H23BrFN5O5S2: [M + H]+ 577.46, found 578.4
- **Compound 6 e:** Light brown solid, (79.56% yield); mp = 112-113 °C; IR: νmax/cm $^{-1}$ = 2947, 2850, 1693, 1593, 1355, 1155; ^{1}H NMR (500 MHz, DMSO):δ 1.99-2.02(2H, m), 2.81-2.85(4H, m), 3.45(8H, Broad), 3.60(8H, Broad), 4.21(2H, Broad), 7.12-7.14(1H, d, *J* = 10Hz),7.35-7.37(1H, d, *J* = 10Hz), 7.43 (1H, s), 7.63 (1H, s); ^{13}C NMR (500 MHz, DMSO): δ 25.48 (1C, s), 32.49 (2C, s), 51.79 (7C, s), 66.71 (2C, s), 119.06 (1C, s), 121.97 (1C, s), 123.59 (1C, s), 124.00 (1C, s), 132.16 (1C, s), 143.42(1C, s), 144.24(1C, s), 146.02(1C, s), 147.12(1C, s), 150.94 (1C, s), 156.77 (1C, s), 162.78 (1C, s); HRMS (ESI) m/z calcd. for C23H29N5O5S2: [M + H] $^+$ 520.6, found 520.1.
- **Compound 6 f:** Light brown solid, (84.80% yield); mp = 115-116 °C ; IR: νmax/cm $^{-1}$ = 2853, 1749, 1343, 1161; ^{1}H NMR (500 MHz, DMSO): δ 2.98 (2H, Broad), 3.57(8H, *Broad*), 3.61 (8H, *Broad*), 7.50-8.14 (8H, m); ^{13}C NMR (500 MHz, DMSO): δ 51.83 (7C, s), 66.69 (2C, s), 123.39 (1C, s), 124.48 (1C, s), 126.73 (1C, s), 127.91 (1C, s), 128.38 (1C, s), 128.91 (1C, s), 129.94 (1C, s), 132.39 (1C, s), 132.62 (1C, s), 133.16 (1C, s), 135.08(1C, s), 144.71 (1C, s), 146.19 (2C, s), 162.90 (1C, s); HRMS (ESI) m/z calcd. for C24H27N5O5S2: [M + H] $^+$ 530.6, found 529.9.
- **Compound 6 g:** Light brown solid, (76.32% yield); mp = 115-116 °C; IR: νmax/cm $^{-1}$ = 2920, 2853, 1621, 1525, 1271; ^{1}H NMR (500 MHz, DMSO): δ 2.47-2.50(4H, *Broad*), 3.12-3.20 (4H, *Broad*), 3.34 (2H, Singlet), 3.60 (8H, *Broad*), 3.90(3H, Singlet), 7.25-7.27(1H, d, *J* = 10Hz), 7.60 (1H, s), 7.79-7.84 (2H, m); ^{13}C NMR (500 MHz, DMSO): δ 46.06 (4C, s), 52.46 (2C, s), 56.94 (1C, s), 59.96 (1C, s), 66.72 (2C, s), 111.60 (1C, s), 116.24 (1C, s), 118.22 (1C, s), 127.91 (1C, s), 132.99 (1C, s), 137.86 (1C, s), 144.64 (1C, s), 156.50 (1C, s), 157.34 (1C, s), 163.05 (1C, s), 169.21 (1C, s); HRMS (ESI) m/z calcd. for C21H26BrN5O5S2: [M + H] $^+$589.5, found 590.
- **Compound 6 h:** Light yellow solid, (85.52% yield); mp = 149-150 °C ; IR: νmax/cm $^{-1}$ = 2919, 2853, 1628, 1526, 1445, 1166; ^{1}H NMR (500 MHz, DMSO): δ 2.62-2.64 (4H, Broad), 2.89 (3H, s), 3.07-3.15(6H, *Broad*), 3.61 (8H, *Broad*), 7.62 (1H, s); ^{13}C NMR (500 MHz, DMSO): δ 34.25 (1C, s), 47.57 (4C, s), 52.08 (2C, s), 59.91 (1C, s), 66.64 (1C, s), 66.80 (1C, s), 118.22 (1C, s), 144.65 (1C, s), 157.37 (1C, s), 163.06 (1C, s), 169.23 (1C, s); HRMS (ESI) m/z calcd. for C15H23N5O5S2: [M + H] $^+$ 418.5, found 418.
- **Compound 6 i:** Light brown solid, (84.25% yield); mp = 124-125 °C; IR: νmax/cm $^{-1}$ = 2922, 2853, 1691, 1628, 1524, 1163; ^{1}H NMR (500 MHz, DMSO):δ 2.59-2.93 (2H, Broad), 3.16 (4H, Broad), 3.38-3.48(8H, *Broad*), 3.60 (4H, *Broad*), 7.60-7.75 (6H, m); ^{13}C NMR (500 MHz, DMSO): δ 41.99 (2C, s), 46.29 (1C, s), 46.36 (1C, s), 51.78 (1C, s), 51.83 (1C, s), 59.84 (1C, s), 66.56 (1C, s), 66.64 (1C, s), 118.15 (1C, s), 128.01 (1C, s), 128.05 (1C, s), 129.90 (1C, s), 133.77 (1C, s), 135.23 (1C, s), 135.30 (1C, s), 144.61 (1C, s), 157.35 (1C, s), 163.06 (1C,s), 169.21 (1C, s); HRMS (ESI) m/z calcd. for C20H25N5O5S2: [M + H] $^+$ 480.6, found 580.3.

- **Compound 6 j:** Off white solid, (73.44% yield); mp = 125-126 °C; IR: νmax/cm $^{-1}$ = 2924, 2854, 1619, 1523, 1154; ^{1}H NMR (500 MHz, DMSO): δ 2.58-2.60 (4H, m), 3.27-3.28 (4H, m), 3.61(10H, Broad), 7.61(1H, s), 8.09-8.18 (2H, 8.09-8.10 (d, *J* = 5Hz), 8.17-8.18(d, *J* = 5 Hz)); ^{13}C NMR (500 MHz, DMSO): δ 45.91 (2C, s), 47.38 (2C, s), 52.21 (2C, s), 59.85 (1C, s), 66.75 (2C, s), 118.18 (1C, s), 130.93 (1C, s), 132.92 (2C, s), 134.18 (2C, s), 136.17 (1C, s), 137.30 (1C, s), 144.63 (1C, s), 157.34 (1C, s), 163.01(1C, s), 169.24 (1C, s); HRMS (ESI) m/z calcd. for C21H24F3N5O5S2: [M + H] $^{+}$ 548.57, found 548.5.

V. 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 (Series 1- S1-S10) 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 w.[32-34]

A. 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).[32-34]

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 compound.

B. 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 (**6a** to **6j**) and the ascorbic acid are measured at different concentrations between 25-400 µg/mL and reported.[35-36]

VI. CONCLUSION

In the present study synthesis, structure elucidation, and biological evaluation of 10 novel sulfonamide derivatives of a hybrid scaffold consisting of Morpholine, Piperazine, and Thiazole are reported. All compounds were purified by either recrystallization or column purification and afforded good yield. A study of their antimicrobial and antioxidant properties disclosed that all compounds can suppress the growth of different strains of bacteria and fugues to varying degrees. On the other hand, ordinary results have been seen for antioxidant activity. Moreover, compound **6e** showed the same activity as the control Standard against Gram-negative strains and fungal strains indicating that indane substitution enhances antimicrobial activity. Furthermore, the substituted phenyl ring showed good activity instead of the Methyl group and unsubstituted phenyl ring, It is seen in compounds **6b** and **6g** that the presence of electron donating groups such as methoxy and tert butyl group at para and ortho position enhances the antimicrobial activity. All these results can be useful for future efforts to synthesize and evaluate sulfonamide derivatives. Additionally, it can also be foreseen that these compounds can be further studied for other microbial microorganisms as well as further functional group modification to selected hybrid scaffold may result in discovery of broad-spectrum antimicrobials.

COMPLIANCE WITH ETHICAL STANDARDS

- **Declaration of interest:** The author (s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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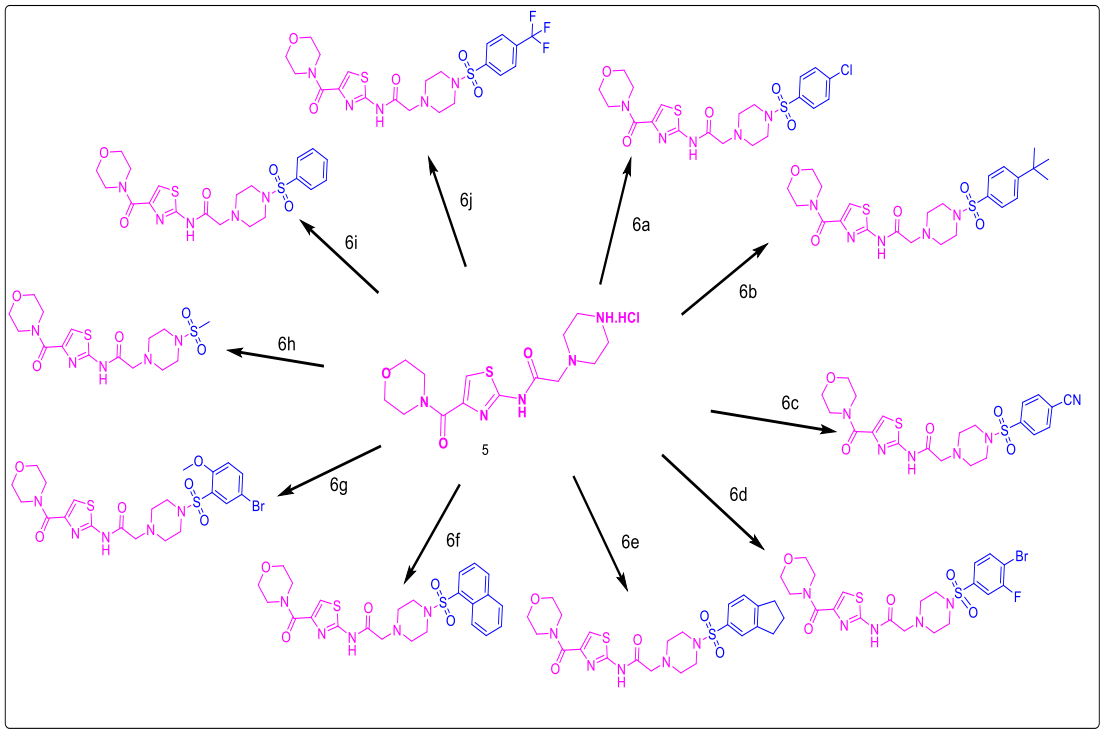

Fig. 4: Synthesized Heterocyclic hybrid compound containing different pharmacophores

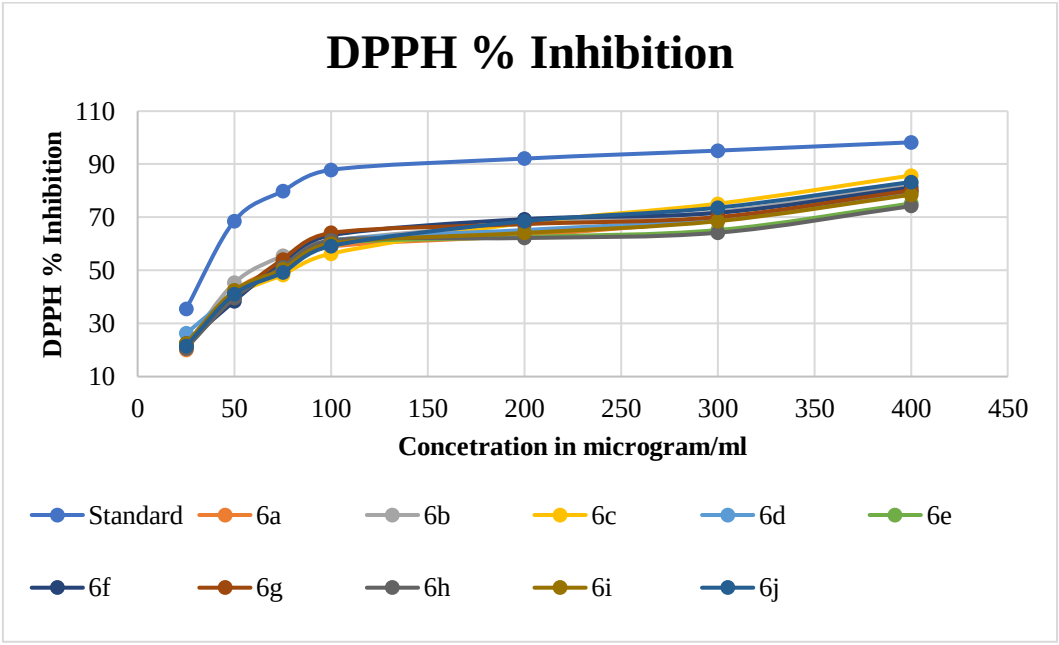

Fig. 5: Effect of Novel hybrids on inhibition of DPPH free radical

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Table 3: 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).

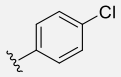
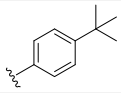
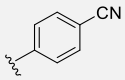
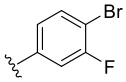
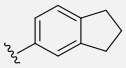
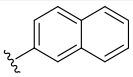
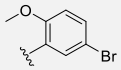
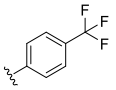
Compound	Substituent R=	Minimum Inhibitory Concentration (MIC) in µg/mL					
		Gram +ve Bacteria		Gram - ve Bacteria		Fungal Microorganism	
		BS	***SA***	***EC***	***PV***	***AN***	***CA***
6a		187.5	250	187.5	500	375	250
6b		250	375	187.5	**250**	375	**187.5**
6c		187.5	250	250	**250**	500	250
6d		187.5	375	250	375	**250**	250
6e		375	250	375	**250**	**250**	**187.5**
6f		187.5	375	187.5	500	375	250
6g		375	250	375	**250**	**250**	250
6h		187.5	250	250	375	375	375
6i		375	250	187.5	375	375	375
6j		187.5	**187.5**	187.5	375	**250**	250
	Standard	**125**	**187.5**	**125**	**250**	**250**	**187.5**

Table 4: Antioxidant activities of compound 6a-j (Values are mean ± SEM of triplicate determinations)

Conc. µg/mL	DPPH % Inhibition										
	Standard	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**
25	35.39	20.14	21.56	22.14	26.24	21.02	22.48	21.07	20.63	22.36	21.48
50	68.64	42.11	45.26	40.21	41.02	39.65	38.45	40.02	39.60	42.36	41.05
75	79.89	51.26	55.42	48.32	51.78	52.36	53.12	54.11	51.22	50.33	49.16
100	87.89	58.95	61.22	56.33	61.21	60.21	63.22	64.11	61.28	60.14	59.22
200	92.13	63.14	67.55	67.89	65.23	62.58	69.33	67.45	62.20	64.11	68.52
300	95.13	69.32	72.04	75.14	69.54	65.21	71.69	70.11	64.20	68.55	73.65
400	98.26	79.55	82.65	**85.75**	79.54	75.22	81.24	80.14	74.21	78.50	**83.32**

- **Scheme-1**: (i) 4M HCl in 1,4 Dioxane, 0°C to rt,12h,(ii) Chloroacetyl chloride, DIPEA, DMF 0°C to rt,4h,(iii) DIPEA, Boc Piperazine,DMF,rt,12h,(iv) 4M HCl in 1,4 Dioxane, 0°C to rt,12h.(v) Respective sulfonyl chloride,DIPEA.DMF,0°C to rt,12h.

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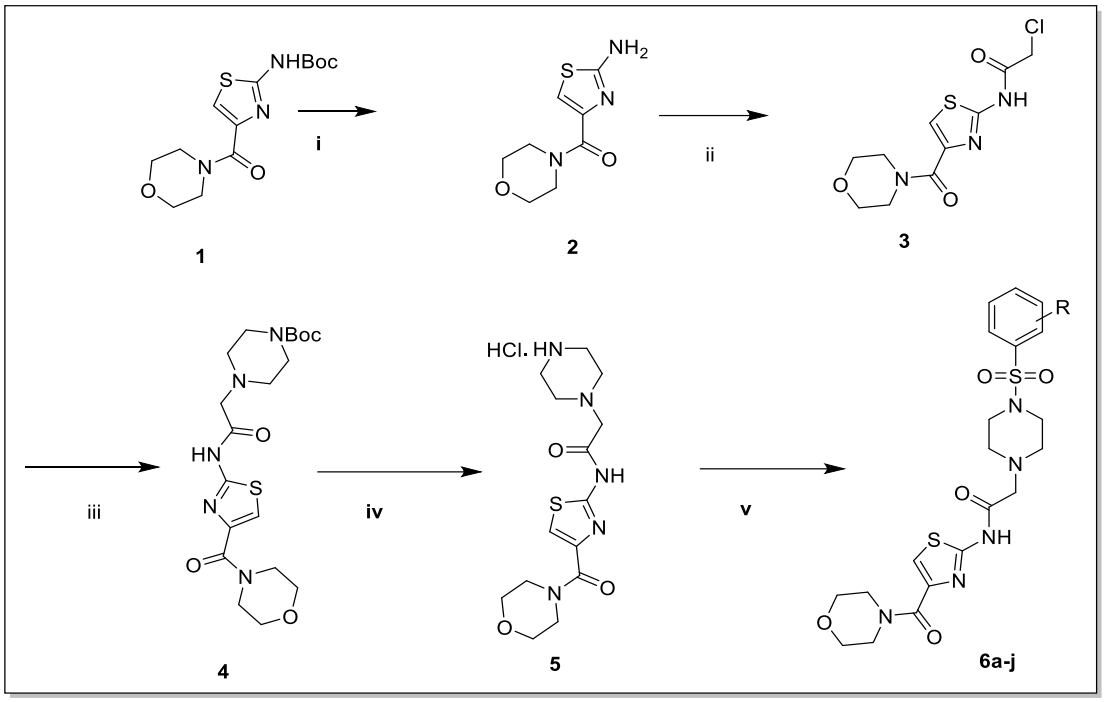

Fig. 6: Chemistry equations