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Synthesis of 1, 2, 3-Triazole conjugate Thiazolidine-2-Thiones heterocyclic derivatives by Click Chemistry and its Biological Activity

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Abstract:
Background*:A novel methodology for the synthesis of thiazolidine -2-thione conjugated 1,2,3-triazole derivatives applied for the first time wherein triazole moiety was coupled with the sulfur containing five-membered ring thiazolidine- 2-thione using Click reaction conditions. The derivatives from 8a-k was synthesized and subjected to microbial activity, out of which four compounds showed good activity(8a, 8b, 8e, 8k).*
Keywords*: Thiazolidine-2-thione, 1,2,3-triazole, Five membered ring, Click reaction, Biological activity*

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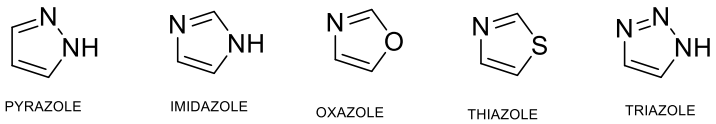
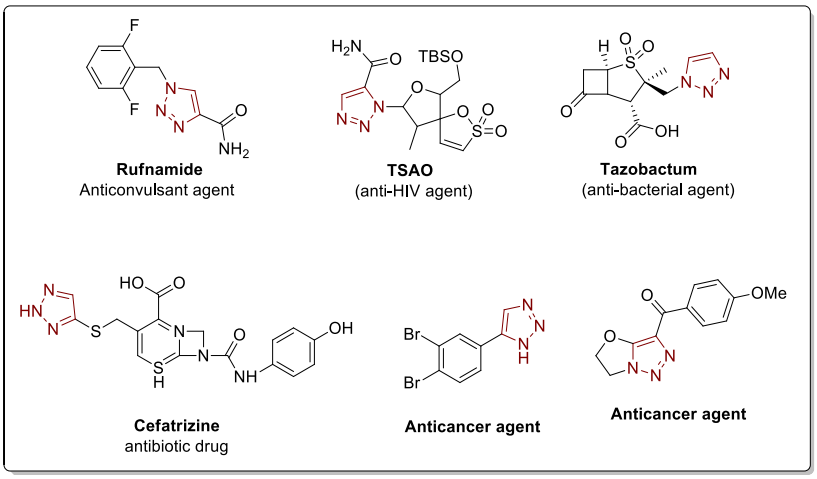
I. Introduction

The well studied and popular branch of medicinal chemistry is the heterocyclic [1] chemistry. It focusses both on theoretical and medicinal importance. Heterocyclic chemistry consists of heteroatoms such as N, O, S along with C atoms. The five membered[2] heterocyclics are derived from Pyran, Furan and Thiophene by replacing the carbon with N, O, S etc. Heterocyclics with more than one or different heteroatom which may be same or different heteroatoms such as pyrazole, Imidazole, Oxazole, Thiazole, Triazole etc. are listed below posses varied activities[3] like antiviral, antitumour, antibacterial, antioxidant, antimicrobial and antituberculosis, etc,.



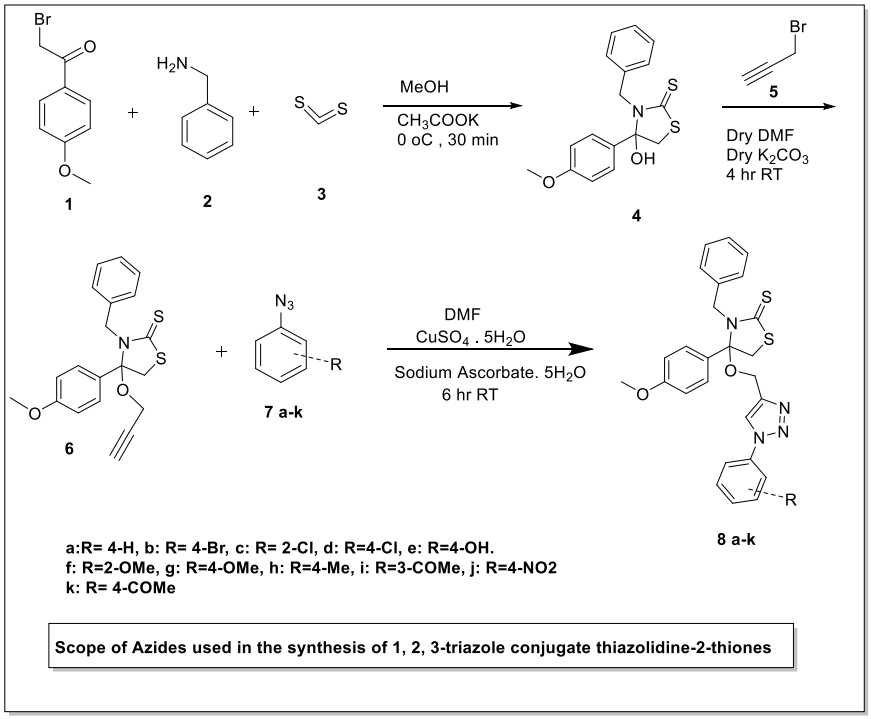
Amongst these the five-membered heterocyclic compounds with three N atoms such as triazole derivatives[4], is the significant organic intermediate showing different organic transformations.Triazole and its hybrids have important implications in medicinal chemistry due to their diverse biological activities. Among these, 1,2,3-triazole is recognized as the most stable and rigid organic moiety compared to other organic moieties containing three adjacent nitrogen atoms. This unique structural feature confers upon triazole hybrids the ability to bind easily with various enzymes and receptors through hydrogen bond interactions, hydrophobic interactions, and vanderWaals interactions. Furthermore, 1,2,3-triazole derivatives can be easily synthesized[5] by simple chemical processes. Literature review has shown that 1,2,3-triazole hybrids exhibit exceptional anticancer activity against many human cell lines, as well as other biological activities such as antifungal, antibacterial, antiallergic, antitubercular, anti-inflammatory activities[6]**Figure1.** preparation of oxazolidinones and thiazolidinones as oxygen and sulphur containing scaffolds are well known modifications as a chiral auxillaries in the field of asymmetric synthesis and coordination chemistry.



(**Figure 1**).**Some of the FDA approved drugs containing 1,2,3-triazole moiety**

II. Material And Methods

The methodology focused on the preparation of triazole compound conjugated to thiazolidine-2-thione. Present procedure highlights the utilization of substituted α- bromo acetophenone **1**, benzyl amine **2**, with carbon disulfide **3** in the presence of potassium acetate and methanol at 0^{O}C for 30 min as a model substrates for the optimization of the reaction conditions for the preparation thiazolidine- 2-thiones **4** . The compound **4** was subjected to reaction with propargyl bromide **5** in the presence of dry dimethyl formamide as solvent and dry potassium carbonate as a base at room temperature maintaining at room temperature to form oxygen protected propargyl thiazolidine -2-thione **6**.Click chemistry[7-10] was applied to the reaction with substituted propargyl substrate with substituted azides**7a-k** to furnish the derivatives of 1, 2, 3-triazole conjugate thiazolidine-2-thiones **8a-k.**



Scheme 1:Representation of 1, 2, 3-triazole conjugate thiazolidine-2-thiones

The design and route for synthesis of compound **8(a-k)** shown in schematic representation (S**cheme 1**). The 1,2,3-triazole conjugate thiazolidine-2-thione core nucleus was constructed from commercially available compounds.Synthesis of starting compounds were carried out 2-bromo-1-(4-methoxyphenyl)ethanone(**1**), phenylmethanamine**(2)** and carbon disulfide **(3)** in the presence of CH_3COOK in MeOH as solvent at 0 ^{o}C for 30 min to obtained compound 3-benzyl-4-hydroxy-4-(4-methoxyphenyl)thiazolidine-2-thione (**4**). Compound (**4**)

react with 3-bromoprop-1-yne (**5**) in dry DMF and dry K_2CO_3 at RT for 4 hrs to afford 3-benzyl-4-(4-methoxyphenyl)-4-(prop-2-yn-1-yloxy)thiazolidine-2-thione**(6)**. Compound (**6**) was condensed with azidobenzene (**7a-k**) in DMF in the presence of $CuSO_4$. $5H_2O$ and Sodium Ascorbate. $5H_2O$ at RT for 6hr to afford 3-benzyl-4-((1-(phenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-(4-methoxyphenyl)thiazolidine-2-thiones**(8a-8k). Figure 1.1**

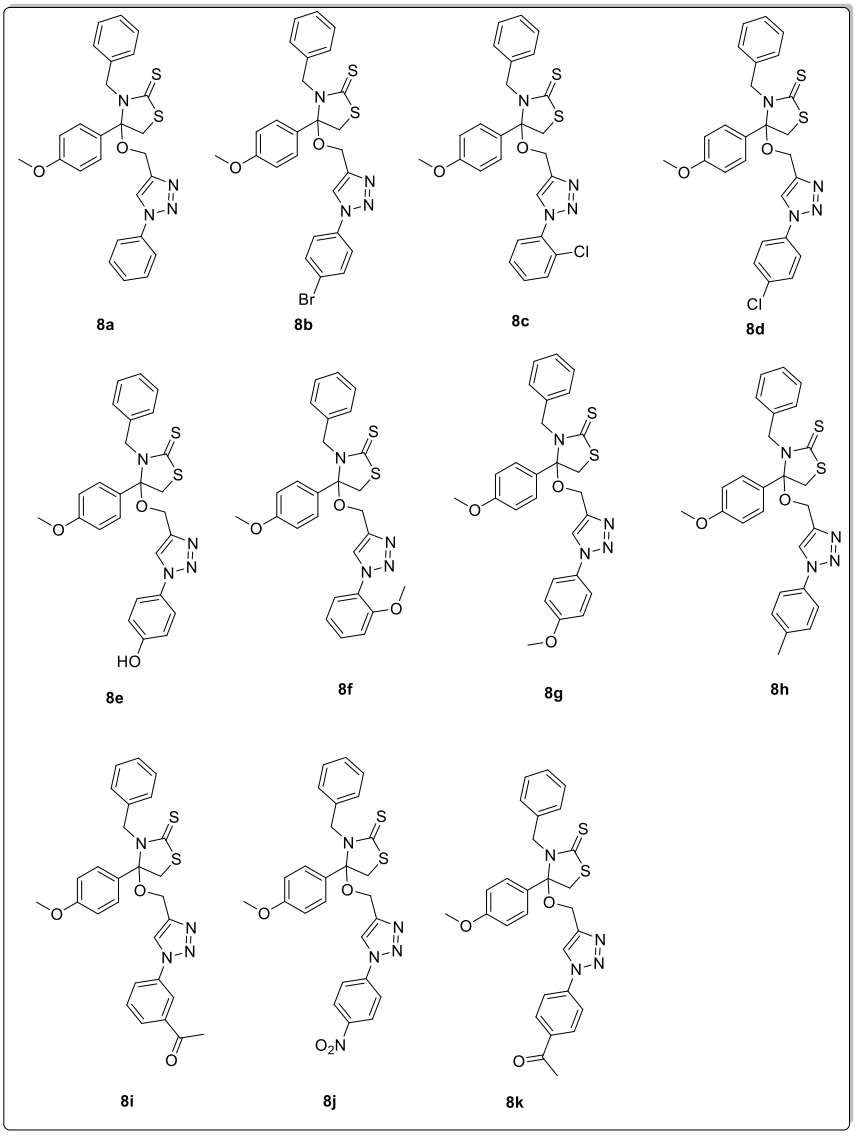


Fig1.1 Derivatives of substitutted 1,2,3-Triazole Thiazolidine -2-Thione

III. Result and Discussions

Experimental Details

General methods

All the chemicals, solvents used in for this work procured form the commercial vendors and used without further purifications. Oven dried Borosil glassware were used. Pre-coated 60G F254 (Merk) silica gel plates were used for TLC monitoring. Melting points were recorded on Electro thermal instrument and mentioned in ^oC. NMR spectra recorded on Bruker Avance-III instrument and TMS is taken as an internal standard. Spin multiplicities mentioned as s (singlet), d (doublet), t (triplet), dd (double doublet), q (quartet). The chemical shifts values mentioned in ppm and coupling constant calculated and mentioned in Hertz. Masses of the compounds recorded by SHIMAZU-LCMS-2010A instrument. Biological applications carried out in sterile conditions and cell lines and dyes purchased form licenced suppliers.

Chemistry

General procedure for the synthesis for 3-benzyl-4-hydroxy-4-(4-methoxyphenyl)thiazolidine-2-thione (**4**).

Charged 2-bromo-1-(4-methoxyphenyl)ethanone (1.1 mmol), phenylmethanamine (2.1 mmol) and carbon disulfide (3.1 mmol) compounds into MeOH (50 ml) solvent in the RBF. Reaction mixture is cooled to 0^oC and added CH_3COOK as catalyst and stirred for 30 min at same temperature. The reaction progress checked with TLC and subjected for flash column (n-hexane:ethyl acetate) solvent system to get pure compound (4).

General procedure for the synthesis for 3-benzyl-4-(4-methoxyphenyl)-4-(prop-2-yn-1-yloxy)thiazolidine-2-thione (**6**).

3-benzyl-4-hydroxy-4-(4-methoxyphenyl)thiazolidine-2-thione (**4**) (0.0028 moles) dissolved in dry DMF solvent and allowed the reaction for stirring after 5-10 min added 3-bromoprop-1-yne (5) (0.40 moles) was added dropwise and continued stirring for 4 hr to afford 3-benzyl-4-(4-methoxyphenyl)-4-(prop-2-yn-1-yloxy)thiazolidine-2-thione (6). The reaction progress checked with TLC and subjected for flash column (n-hexane:ethyl acetate) solvent system to get pure compound.

General procedure for the synthesis for 3-benzyl-4-(4-methoxyphenyl)-4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)thiazolidine-2-thione (8 a-k).

A mixture of 3-benzyl-4-(4-methoxyphenyl)-4-(prop-2-yn-1-yloxy)thiazolidine-2-thione (6) (2.57 moles) dissolved in dimethylformamide solvent and allowed to stir the reaction mixture. After a few minutes added arylazides (7a-k) after that CuSO4.5H2O catalyst was added and sodium ascorbate as a reducing agent to obtain 1,3-dipolar cycloaddition product. The progress of the reaction confirmed with thin layer chromatography and target compound purified by column chromatography using n-hexane:ethyl acetate solvent system to afford pure

3-benzyl-4-(4-methoxyphenyl)-4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)thiazolidine-2-thione **8** (a-k).

Spectral data:

3-benzyl-4-(4-methoxyphenyl)-4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)thiazolidine-2-thione 8a:

M.P: 180-182^{o}C; Yield: 59%; Pale Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.60 (s, 1H), 7.59 – 7.53 (m, 2H), 7.52 – 7.44 (m, 2H), 7.42 – 7.20 (m, 8H), 6.90 (d, 2H), 5.19 (s, 2H), 4.94 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, δ; ppm): δ 195.74, 159.73, 147.44, 138.39, 136.54, 134.16, 130.07, 128.61, 128.18, 127.79, 127.61, 120.83, 118.11, 113.57, 97.47, 55.36, 54.21, 48.15, 41.90; LCMS m/z calcd for $C_{26}H_{24}N_4O_2S_2$:488.13; found [M+1] 489.

3-benzyl-4-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-(4-methoxyphenyl) thiazolidine-2-thione 8b:

M.P: 184-187^{o}C; Yield: 51%; Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.60 (s, 1H), 7.70 – 7.58 (m, 4H), 7.36 – 7.20 (m, 7H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.77, 159.76, 147.47, 138.42, 135.36, 134.19, 133.74, 128.64, 128.21, 127.82, 121.62, 119.75, 118.26, 113.60, 97.50, 55.39, 54.24, 48.18, 41.93; LCMS m/z calcd for $C_{26}H_{23}N_4O_2S_2Br$:567.52;found [M+1] 568.

3-benzyl-4-((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-(4-methoxyphenyl) thiazolidine-2-thione 8c:

M.P: 190-192^{o}C; Yield: 55%; Yellowish White Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.53 (s, 1H), 7.76 (d, *J* = 1.5 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.40 – 7.20 (m, 8H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.75, 159.74, 147.65, 138.40, 135.86, 134.17, 131.64, 128.87, 128.63, 128.21, 128.19, 127.84, 127.80, 121.05, 119.17, 113.58, 97.48, 55.37, 54.39, 48.16, 41.91; LCMS m/z calcd for $C_{26}H_{23}N_4O_2S_2Cl$:522.10;found [M+1] 524.

3-benzyl-4-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-(4-methoxyphenyl) thiazolidine-2-thione 8d:

M.P: 192-194^{o}C; Yield: 56%; Yellowish White Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.61 (s, 1H), 7.76 – 7.70 (m, 2H), 7.48 – 7.42 (m, 2H), 7.36 – 7.20 (m, 7H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.74, 159.73, 147.44, 138.39, 135.03, 134.76, 134.16, 130.35, 128.62, 128.18, 127.79, 122.21, 118.27, 113.57, 97.47, 55.36, 54.21, 48.15, 41.90. LCMS m/z calcd for $C_{26}H_{23}N_4O_2S_2Cl$:522.10;found [M+1] 524.

3-benzyl-4-((1-(4-hydroxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)-4-(4-methoxyphenyl) thiazolidine-2-thione 8e

M.P: 179-181ºC; Yield: 54%; Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 9.12 (s, 1H), 8.59 (s, 1H), 7.72 – 7.66 (m, 2H), 7.36 – 7.20 (m, 7H), 6.97 – 6.87 (m, 4H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.73, 159.72, 156.79, 147.43, 138.38, 134.15, 129.75, 128.60, 128.17, 127.78, 122.07, 118.42, 117.42, 113.56, 97.46, 55.35, 54.20, 48.14, 41.89. LCMS m/z calcd for $C_{26}H_{23}N_4O_2S_2OH$:504.62; found [M+1] 505.

3-benzyl-4-(4-methoxyphenyl)-4-((1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy) thiazolidine-2-thione 8f:

M.P: 175-177ºC; Yield: 58%; Pale Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.38 (s, 1H), 7.72 (d, *J* = 1.4 Hz, 1H), 7.40 (dd, *J* = 7.1, 1.1 Hz, 1H), 7.35 – 7.25 (m, 5H), 7.25 – 7.21 (m, 2H), 7.18 (dd, *J* = 7.1, 1.4 Hz, 1H), 7.05 (d, *J* = 1.3 Hz, 1H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.84 (s, 3H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.76, 159.75, 153.91, 147.50, 138.41, 134.18, 128.64, 128.20, 127.81, 127.29, 126.90, 124.91, 119.76, 118.80, 114.08, 113.59, 97.49, 55.76, 55.38, 54.40, 48.17, 41.92; LCMS m/z calcd for $C_{26}H_{23}N_4O_2\,S_2OCH_3$:518.65;found [M-1]=516.

3-benzyl-4-(4-methoxyphenyl)-4-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy) thiazolidine-2-thione 8g:

M.P: 172-174ºC; Yield: 58%; Pale Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.58 (s, 1H), 7.66 – 7.59 (m, 2H), 7.36 – 7.20 (m, 7H), 7.06 – 6.99 (m, 2H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 6H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.74, 159.89, 159.73, 147.44, 138.39, 134.16, 130.84, 128.61, 128.18, 127.79, 122.15, 118.43, 115.36, 113.57, 97.47, 55.36, 54.21, 48.15, 41.90; LCMS m/z calcd for $C_{27}H_{26}N_4O_3S_2$:518.65;found [M-1]=516.

3-benzyl-4-(4-methoxyphenyl)-4-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methoxy)thiazolidine-2-thione 8h:

M.P: 184-186ºC; Yield: 57%; Yellow Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.60 (s, 1H), 7.67 – 7.61 (m, 2H), 7.36 – 7.20 (m, 9H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.77, 159.76, 147.47, 138.42, 137.22, 134.77, 134.19, 130.58, 128.65, 128.21, 127.82, 120.37, 118.13, 113.60, 97.50, 55.39,54.24,48.18,41.93, 21.12; LCMS m/z calcd for $C_{27}H_{26}N_4O_2S_2$:502.65;found [M+1] 503.

1-(3-(4-(((3-benzyl-4-(4-methoxyphenyl)-2-thioxothiazolidin-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone 8i:

M.P: 186-188ºC; Yield: 50%; Yellowish Green Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.65 (s, 1H), 8.15 (s, 1H), 7.83 (d, *J* = 1.3 Hz, 1H), 7.68 (d, *J* = 1.4 Hz, 1H), 7.59 (dd, *J* = 7.3, 7.3 Hz, 1H), 7.36 – 7.20 (m, 7H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H), 2.62 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 197.54, 195.77, 159.76, 147.48, 138.42, 138.28, 137.65, 134.19, 129.89, 128.65, 128.21, 127.82, 126.23, 122.18, 119.47, 118.41, 113.60, 97.50, 55.39, 54.24, 48.18, 41.93, 26.75. LCMS m/z calcd for $C_{27}H_{26}N_4O_3S_2$:530.14; found [M+1] 531.

3-benzyl-4-(4-methoxyphenyl)-4-((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl)methoxy) thiazolidine-2-thione 8j:

M.P: 192-194ºC; Yield: 50%; Yellowish Green Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.63 (s, 1H), 8.37 – 8.30 (m, 2H), 8.00 – 7.94 (m, 2H), 7.36 – 7.20 (m, 7H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 195.77, 159.76, 149.67, 147.47, 139.61, 138.42, 134.19, 128.65, 128.21, 127.82, 126.05, 121.26, 118.25, 113.60, 97.50, 55.39, 54.24, 48.18, 41.93. LCMS m/z calcd for $C_{26}H_{23}N_5O_4S_2$:533.62; found [M+1] 535.

1-(4-(4-(((3-benzyl-4-(4-methoxyphenyl)-2-thioxothiazolidin-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone 8k

M.P: 185-187ºC; Yield: 50%; Yellowish Green Solid;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.66 (s, 1H), 7.95 – 7.89 (m, 2H), 7.77 – 7.71 (m, 2H), 7.36 – 7.20 (m, 7H), 6.93 – 6.87 (m, 2H), 5.06 (s, 2H), 4.81 (s, 2H), 3.76 (s, 3H), 3.36 (s, 2H), 2.56 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 196.77, 195.74, 159.73, 147.44, 138.39, 138.36, 135.43, 134.16, 130.62, 128.62, 128.18, 127.79, 120.10, 118.23, 113.57, 97.47,55.36,54.21,48.15, 41.90, 26.42. LCMS m/z calcd for $C_{27}H_{26}N_4O_3S_2$:530.14; found [M+1] 531.

Biological Activity

Antibacterial activity and MIC assay of given compounds against Gram positive and Gram negative bacteria

Methodology:

Requirements:

- Active cultures of Gram positive and Gram negative bacteria, fungal cultures
- Nutrient agar media, Potato Dextrose Agar media, Yeast Extract Peptone media
- Petri plates
- Bacterial incubator
- Routine lab equipment's
- Standard – streptomycin (5 µg/mL)

Procedure:

Preparation of active bacterial cultures – A single bacterial colony of pure culture is transferred into a 150 ml conical flask containing 50 ml nutrient broth media and incubated for 8-12hrs at 37 °C.

Preparation of Sample concentrations: In case of powdered compounds, samples were dissolved in 1ml of suitable solvent like water/methanol/DMSO etc and made into aliquots of different concentrations for MIC assay. Liquid samples were used directly and are diluted using water/solvent based on required concentrations.

Antibacterial assay: The antibacterial assay was carried out by performing pour plate method in which 1% of active bacterial cultures were mixed into autoclaved agar media just before solidifying temperature and poured into the plates. Here Staphylococcus aureus and Pseudomonas aeruginosa were selected as Gram positive and Gram negative bacteria respectively.

After the plates were solidified, wells were made using sterile well borer and samples were loaded 100µl each into the wells respectively. Plates were incubated at 37 °C for 18-24 hours in a bacterial incubator.

Interpretation of results: The bacterial plates were observed after incubation period and following results were noted:

(**NOTE:** The zone of inhibition is measured from one end to other end of the clear inhibition zone i.e. zone of clearance. if the growth towards well is good (less mm), compound is having less inhibitory activity, and if the growth is less (more mm) compound has good inhibition activity)

S.no	Sample Name	Gram Positive Bacteria inhibition zone (mm)	Gram Negative Bacteria inhibition zone (mm)
		Staphylococcus aureus	**Pseudomonas aeruginosa**
1	**8a**	10mm	10mm
2	**8b**	12mm	08mm
3	**8d**	No activity	No activity
4	**8e**	10mm	10mm
5	**8f**	No activity	No activity
6	**8h**	No activity	No activity
7	**8i**	No activity	No activity
8	**8j**	No activity	No activity
9	**8k**	08mm	08mm
10	standard	15mm	12mm

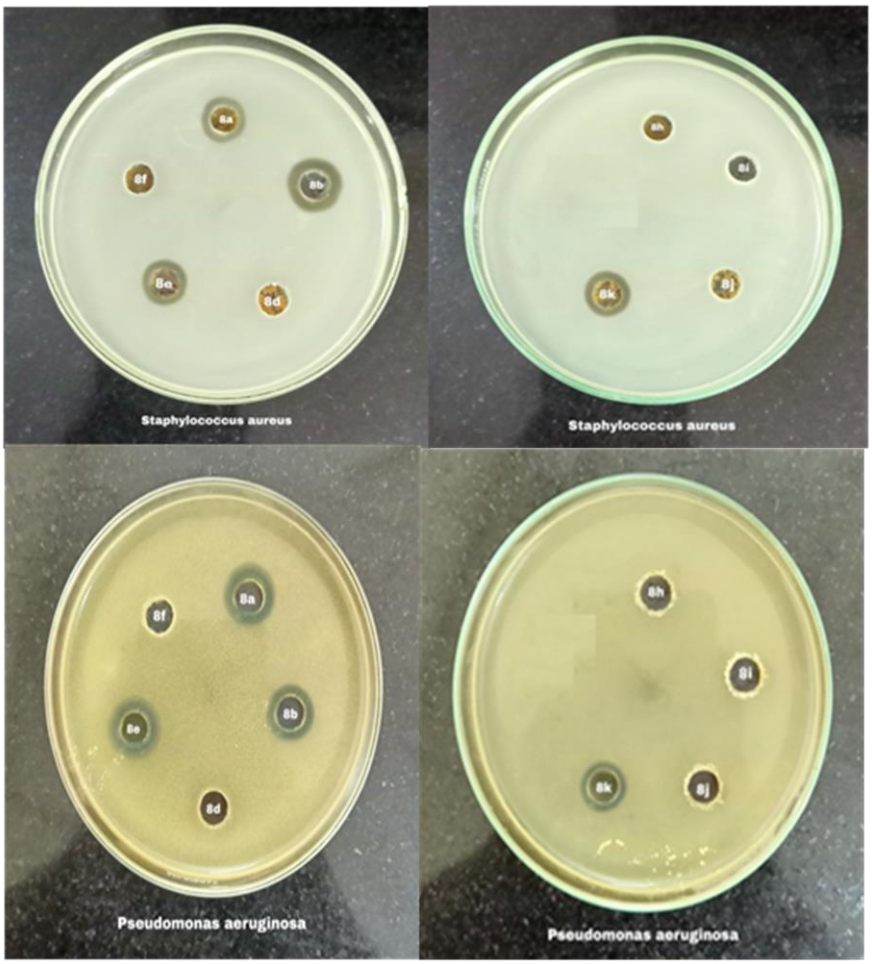


Fig 1.2: Inhibition zone of gram positive and gram negative bacteria of a given samples

The samples checked for antibacterial activity were assayed for their minimum inhibitory concentrations (MIC) by loading 25 µl, 50 µl, 75 µl, 100 µl of samples diluted with water/solvent like DMSO to make up the volume up to 100 µl in each well of the plate respectively. Ex if the concentration of sample is 10 mg/mL, 25µl sample+75 µl solvent will make it 2.5 mg/mL, 50µl sample+50µl solvent = 5 mg/mL, 75µl sample+25 µl solvent = 7.5 mg/mL and 100µl sample = 10 mg/mL concentration respectively. Therefore, samples in each well are diluted accordingly to the required concentrations based on volume ratios of sample and solvent. The MIC were designated as 25, 50, 75, 100 which should be referred for respective dilutions based on sample concentrations as different samples have different concentrations.

S.no	Sample ID	MINIMUM INHIBITORY CONCENTRATION – MIC (mm)				MIC of sample (µL)
		Staphylococcus aureus				
		25µL	50µL	75µL	100µL	
1	**8a**	-	-	-	12mm	100µL
2	**8b**	-	05mm	06mm	10mm	50µL
3	**8d**	-	-	-	-	No activity
4	**8e**	-	-	-	06mm	100µL
5	**8f**	-	-	-	-	No activity
6	**8h**			-	-	No activity
7	**8i**	-	-	-	-	No activity
8	**8j**	-	-	-	-	No activity
9	**8k**	-	-	08mm	10mm	75µL

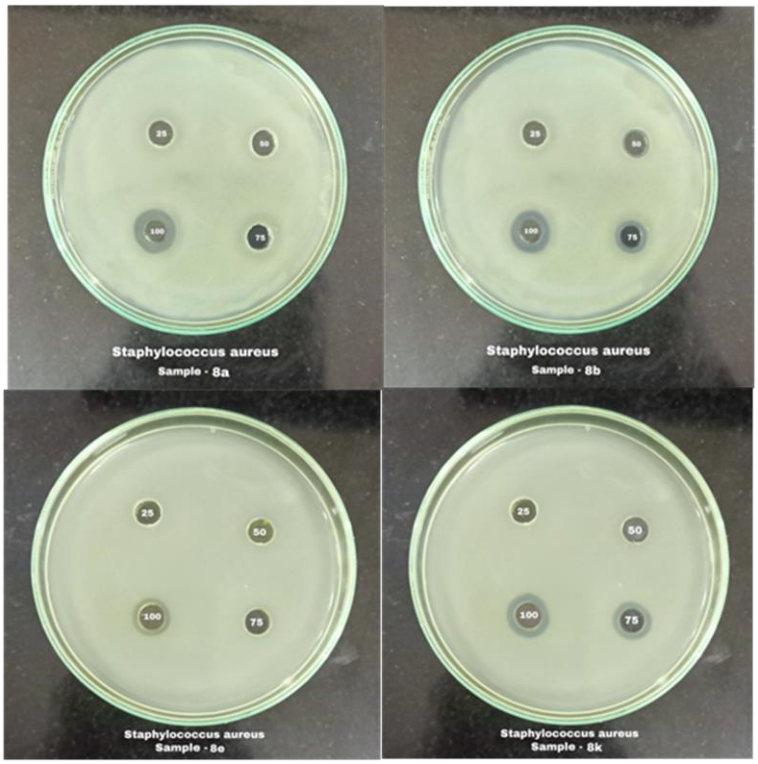


Fig 1.3: Minimum inhibitory concentration (MIC) assay of given samples

S.no	Sample ID	MINIMUM INHIBITORY CONCENTRATION – MIC (mm)				MIC of sample (µL)
		Pseudomonas aeruginosa				
		25µL	50µL	75µL	100µL	
1	**8a**	-	-	06mm	12mm	75µL
2	**8b**	06mm	08mm	10mm	10mm	25µL
3	**8d**	-	-	-	-	No activity
4	**8e**	06mm	08mm	08mm	10mm	25µL
5	**8f**	-	-	-	-	No activity
6	**8h**	-	-	-	-	No activity
7	**8i**	-	-	-	-	No activity
8	**8j**	-	-	-	-	No activity
9	**8k**	06mm	08mm	08mm	10mm	25µL

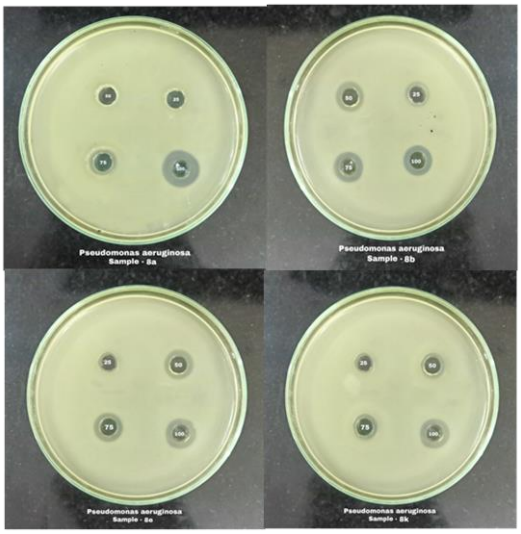


Fig 1.4: Minimum inhibitory concentration (MIC) assay of given samples

Antifungal activity and MIC assay of given compounds against different fungi
Methodology:
Requirements:
Fungal cultures

- Potato dextrose agar
- Yeast Extract Peptone agar
- Petri plates
- Fungal incubator
- Routine lab equipment's
- Standard – Mancozeb WP 75

Procedure:

Antifungal assay: The antifungal assay was performed by using Candida and Aspergillus. Potato dextrose agar and Yeast Extract Peptone agar media were prepared and autoclaved. Just before pouring into the plates, antibiotic (Streptomycin/Chloramphenicol) was added into media to avoid bacterial contamination. The plates were allowed to solidify and 5mm wells were made using sterile well borer based on number of samples. The wells were loaded with 100µl of samples each. The plates were incubated at 25°C for 96 hours and results were noted

Interpretation of results: The fungal plates were observed after incubation time and following results were noted

(**NOTE:** In case of pour plate method where fungal spores of Aspergillus niger are used, the zone of inhibition is measured from one end to other end of the clear inhibition zone i.e zone of clearance. if the growth towards well is good (less mm), compound is having less inhibitory activity, and if the growth is less (more mm) compound has good inhibition activity. In case of fungi with mycelia growth like Fusarium oxysporum, zone of inhibition is measured from edge of fungal plug up to the end/edge of fungal mycelial growth i.e. if the fungal growth towards well is good (more mm), compound is having less inhibitory activity, and if the growth is less (less mm) compound has good inhibition activity)

Result:

S.No	Sample Name	Fungal pathogens inhibition zone (mm)	
		Fusarium oxysporum	Aspergillus niger
1	**8a**	No activity	No activity
2	**8b**	No activity	No activity
3	**8d**	No activity	No activity
4	**8e**	No activity	No activity
5	**8f**	No activity	No activity
6	**8h**	No activity	No activity
7	**8i**	No activity	No activity
8	**8j**	No activity	No activity
9	**8k**	No activity	No activity
10	Standard	16mm	14mm

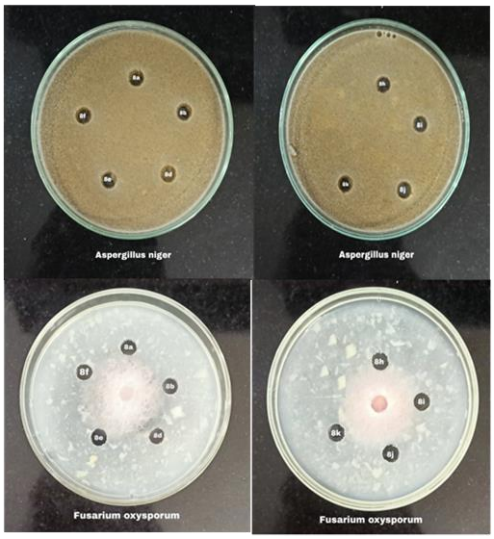


Fig 1.5: Minimum inhibitory concentration (MIC) assay of given samples

The samples checked for antifungal activity were assayed for their minimum inhibitory concentrations (MIC) by loading 25 µl, 50 µl, 75 µl, 100 µl of samples diluted with water/solvent to make up the volume up to 100 µl in each well of the plate respectively.

(**NOTE:** If the concentration of sample is 10 mg/mL, 25µl sample + 75 µl solvent will make it 2.5 mg/mL, 50µl sample + 50µl solvent = 5 mg/mL, 75µl sample+25 µl solvent = 7.5 mg/mL and 100µl sample = 10 mg/mL concentration respectively. Therefore, samples in each well are diluted accordingly to the required concentrations based on volume ratios of sample and solvent. The MIC were designated as 25, 50, 75, 100 which should be referred for respective dilutions based on sample concentrations as different samples have different concentrations)

Result: The samples didn't show any antifungal activity towards both Aspergillus niger and Fusarium oxysporum

IV. Conclusion

In summary, we have designed and synthesized a series of3-benzyl-4-(4-methoxyphenyl)-4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)thiazolidine-2-thiones. Further, screened for in vitro antibacterial and fungus activities.

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