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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 4-((SUBSTITUTED BENZYLIDENE)AMINO)-5-(PYRIDIN-4-YL)-4H-1,2,4-TRIAZOLE-3-THIOL DERIVATIVES AS POTENTIAL ANTICANCER, ANTIMICROBIAL AND ANTI-INFLAMMATORY AGENTS

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ABSTRACT
The anticancer, antimicrobial and anti-inflammatory properties of a novel series of 4-((substituted benzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol derivatives were synthesized and analysed. Using glacial acetic acid as a catalyst, 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol was condensed with a variety of substituted benzaldehydes in ethanol to yield the target compounds. IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry were used to confirm the chemical structures of the resulting derivatives. Numerous compounds showed promising multi-targeted biological profiles, according to biological evaluation. With the strongest anticancer activity and a low IC50 value, compound 7f showed great cytotoxic efficacy. In the albumin denaturation test, compound 7b demonstrated the highest percentage inhibition and outstanding anti-inflammatory activity. Furthermore, antimicrobial investigations revealed that 7e had remarkable antifungal activity against Candida albicans, whereas 7b shown notable zones of inhibition against Escherichia coli and Staphylococcus aureus. The results suggest that the biological activity profile is significantly impacted by the addition of various substituents to the benzylidene molecule. The study indicates that the 1,2,4-triazole-3-thiol scaffold is a potential basic component for the creation of multipurpose medicinal drugs with strong anti-inflammatory, anti-microbial, and anti-cancer effects.

KEYWORDS: 1,2,4-Triazole, Schiff bases, Pyridinyl derivatives, Anticancer; Antimicrobial, Anti-inflammatory.

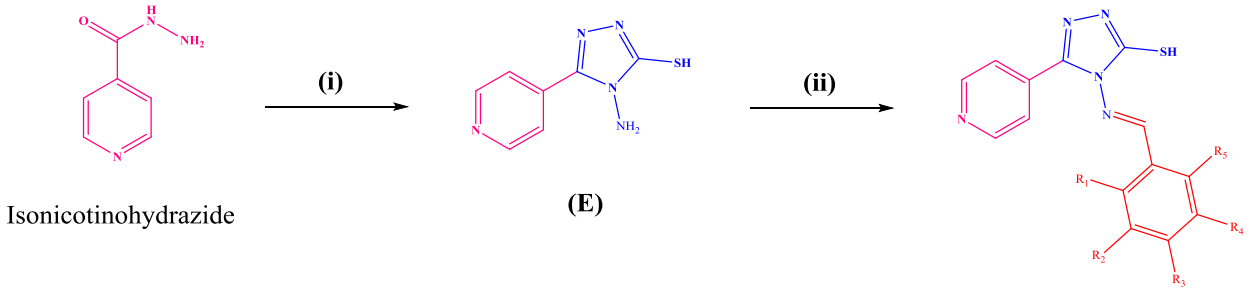
1. INTRODUCTION
With millions of deaths annually, cancer continues to rank among the world's major causes of illness and mortality. Many anticancer drugs have serious side effects, poor selectivity, and resistance development despite tremendous advancements in chemotherapy and tailored drug delivery. As a result, one of medicinal chemistry's main goals is still to find new small compounds with better safety and efficacy profiles. The 1,2,4-triazole nucleus has become a prominent scaffold among heterocyclic frameworks because of its exceptional pharmacological variety and capacity to interact with a wide range of biological targets.[1–3] It is well-known that the 1,2,4-triazole moiety possesses a wide range of biological behaviours, including as antiviral, antitubercular, anticancer, antibacterial, and anti-inflammatory qualities. It is an important component of modern drug design because to its stability, chemical

flexibility and biologically similarity to amide and imidazole groups.[4–8] Triazole rings are found in many clinically approved medicines, including letrozole, voriconazole, and fluconazole, indicating the pharmacophoric importance of this heterocycle.[9–10] Triazole-based compounds in particular have demonstrated the ability to start cell death, inhibit important enzymes implicated in the rise of cancer, and impact cellular signalling pathways, all of which offer incredible potential as anticancer drugs.[11–14] Recent studies have demonstrated the strong cytotoxic and antibacterial properties of thio-substituted triazoles, particularly 4H-1,2,4-triazole-3-thiols.[15–18] Through metal chelation and hydrogen bonding, the thiol group improves flexibility and makes interactions with biological macromolecules easier. Enhancing biological activity through structural hybridization of triazoles with imine linkages produced from aromatic aldehydes has proven to be an effective strategy. Because of their capacity to interact with transition metals and affect redox processes within cells, Schiff base derivatives with azomethine (-CH=N-) activity are known to display a variety of pharmacological actions.[19–21] Additional hydrogen-bonding capacity and electrical modification are introduced by the inclusion of a pyridin-4-yl substituent at the triazole ring's 5-position, potentially improving biological affinity.[22–24] moreover, the benzylidene moiety's pharmacological behaviour and receptor binding are significantly impacted by the addition of various substituents. While electron-donating substituents like -OH or $–OCH_3$ can enhance antioxidant and antibacterial potential, electron-withdrawing groups like -Cl and $–NO_2$ are frequently linked to higher anticancer activity. The logical design of multifunctional compounds is based on these structure–activity relationships (SAR).[25–27] In addition to their anticancer properties, inflammation and microbial infection are frequently connected to the development and spread of tumours. An environment that is supportive to the development of cancer can be created by chronic inflammation, which can cause oxidative stress, DNA damage, and excessive cell growth. Similarly, through pathways involving immune regulation and chronic inflammation, microbial infections have been linked to cancer growth.[28–30] Because they can provide significant medicinal benefits and enhanced clinical results, substances with dual or triple biological activity-anticancer, antibacterial, and anti-inflammatory are of significant interest. We offer a new set of ten 4-((substituted benzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol derivatives, including their design, synthesis, characterisation, and biological evaluation. By adding different electron-donating and electron-withdrawing substituents to the benzylidene moiety, the synthesis method was designed to promote structural variety. To verify their chemical structures, the produced compounds were analysed by mass spectrometry, IR, ^{1}H NMR, and ^{13}C NMR. In addition to antibacterial and anti-inflammatory screening to investigate their multifunctional pharmacological scope, the biological evaluation was conducted to evaluate their anticancer potential using the MTT assay against HeLa and MCF-7 cell lines. The design, synthesis, characterisation, and biological assessment of a novel series of ten 4-((substituted benzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol derivatives are shown in this work. The purpose of the synthetic approach was to add structural variety by adding different substituents to the benzylidene moiety that donate and withdraw electrons. To verify the chemical structures of the produced compounds, IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry were used. Using the MTT assay against HeLa and MCF-7 cell lines, the biological evaluation was conducted to determine their anticancer potential. Antimicrobial and anti-inflammatory screening were also conducted to investigate their multifunctional therapeutic potential.

2. RESULTS AND DISCUSSION

2.1. chemistry

The target compounds (7a-7j) were successfully synthesized via the condensation of 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol with various substituted benzaldehydes in ethanol using glacial acetic acid as catalyst[31] (Scheme 1). The reactions proceeded smoothly under reflux to afford Schiff base derivatives in good yield. The physical and analytical data of the synthesized compounds are summarized in Table 1.



Isonicotinohydrazide

(E)

(7a-j)

(i) TCH, fusion, 120 °C, 5h

(ii) substituted benzaldehude, acetic acid, BMIM[BF_4], MW 500 W, 80-90 °C, 5-7 min

Scheme 1: Synthesis of 4-((substituted benzylidene) amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol.

Table 1: Physical and analytical data of synthesized compounds (7a-7j).

Compound	Substituent on benzylidene ring	Reaction time (h)	Yield (%)	Melting point (°C)	Appearance
7a	2- NO_2	5.5	84	214-216	Yellow-orange solid
7b	4- NO_2	5.0	86	210–212	Yellow solid
7c	4-Cl	5.0	84	202–204	Light yellow solid
7d	2-OH	4.5	80	190-192	Cream solid
7e	4-OCH_3	4.0	75	182-184	Cream crystals
7f	3-Br	4.5	80	210-212	Yellow solid
7g	4-OH	4.0	79	185–187	Cream solid
7h	2-Cl	5.5	88	208–210	Pale orange solid
7i	4- CH_3	4.5	78	188–190	Pale yellow solid
7j	4-Br	5.0	83	206–208	Yellow-orange solid

Spectroscopic data confirmed the successful formation of azomethine (–CH=N–) linkages and triazole-thiol functionalities. The IR spectra showed characteristic C=N stretching at 1610–1630 cm^{-1}, while ^1H NMR spectra exhibited singlets around δ 8.4–8.7 ppm for the imine proton and δ 11.0-11.5 ppm for the thiol proton. The ^{13}C NMR displayed resonances corresponding to azomethine carbon (δ 165-170 ppm) and aromatic carbons (δ 120–155 ppm). Mass spectra gave molecular ion peaks consistent with proposed structures.

2.2. Biological Evaluation

2.2.1. Anticancer Activity

The in vitro cytotoxicity of the synthesized compounds was evaluated using the **MTT assay** on the **HeLa (human cervical cancer)** cell line.[32] Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 atmosphere. Cells (1×10^4 cells/well) were seeded in a 96-well plate and treated with different concentrations (1–100 µM) of the test compounds for 24 hours. After incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. The IC50 values were calculated with **compound 7f (3-Br)** showing the most potent activity. The target compounds (7a-7j) were successfully synthesized through the condensation of 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol with a series of substituted benzaldehydes in ethanol using glacial acetic acid as a mild acid catalyst (Scheme 1). The reaction mixture was refluxed for 4-5 hours during which time the colour gradually changed indicating the formation of Schiff base linkages. Upon completion as monitored by TLC the reaction mass was cooled and the solid product was filtered, washed thoroughly with cold ethanol and recrystallized to afford analytically pure triazole-based Schiff base derivatives. The yields were generally high (70–88%) confirming the efficiency of the condensation process under these optimized conditions. The use of glacial acetic acid proved essential for facilitating imine formation while preventing undesirable oxidation of the thiol group. The physical properties such as melting point, colour and appearance were consistent with formation of new conjugated systems. All compounds displayed sharp melting points, indicating purity. The general synthetic procedure demonstrated excellent reproducibility and adaptability to a wide range of aromatic aldehydes bearing electron-donating or electron-withdrawing substituents. These findings establish an efficient and environmentally gentle route for constructing novel 1,2,4-triazole-3-thiol–based Schiff bases suitable for further biological evaluation.

2.2.2. Antimicrobial Activity

Antimicrobial activity was evaluated by the **disc diffusion method** against selected bacterial and fungal strains:

- **Gram-negative bacteria:** *Escherichia coli*, *Pseudomonas aeruginosa*
- **Gram-positive bacteria:** *Staphylococcus aureus*, *Bacillus cereus*
- **Fungal strain:** *Candida albicans*

Antimicrobial screening of the synthesized compounds was performed using the disc diffusion method to evaluate their efficacy against representative bacterial and fungal strains.[33,37,38] A saline suspension of bacterial culture was prepared from overnight incubated agar plate (As per 0.5 McFarland turbidity standard. MHA plates were prepared and inoculated with test organisms using a sterile swab. Sterile empty discs were placed on inoculated plates and 30 microlitre of compounds with concentrations from 20mg to 1.25mg/ml were loaded on the discs. Three positive controls ampicillin, tetracycline and gentamicin, solvent control DMSO and water as negative control were also placed. Then plates were incubated at 37°C for 24 -48 hrs. After incubation the zone was measured with the help of zone measuring scale. Among all tested derivatives, compounds **7b (4-NO_2)** and **7e (4-OCH_3)** exhibited the most significant antibacterial and antifungal activities respectively which indicating that the presence of nitro and methoxy substituents enhances the biological affinity. The results highlight the potential of these triazole–Schiff base hybrids as promising antimicrobial leads for further optimization and development.[34,35,38]

2.2.3. Anti-Inflammatory Activity

The anti-inflammatory potential of the synthesized compounds were assessed by the **egg albumin denaturation assay** which is a well-established in vitro model for evaluating the ability of compounds to prevent protein denaturation, a key process involved in inflammatory responses.[36] Test samples at concentrations ranging from **100 to 500 µg/mL** were mixed with 0.2 mL of freshly prepared egg albumin and phosphate-buffered saline (pH 6.4). The mixtures were incubated at **37 °C for 20 minutes**, followed by heating at **70 °C for 5 minutes** to induce controlled denaturation. After cooling to room temperature, absorbance was recorded at **660 nm**, and the **percentage inhibition of protein denaturation** was calculated relative to a control sample containing only buffer and albumin. **Diclofenac sodium** served as the reference anti-inflammatory agent, ensuring accurate comparative analysis. Among all derivatives, **compound 7b (4-Cl)** exhibited the highest inhibition percentage, indicating potent anti-inflammatory activity likely due to enhanced lipophilicity and effective stabilization of protein structures under stress conditions. The results suggest that substitution with halogen atoms significantly contributes to anti-inflammatory efficacy in triazole–Schiff base hybrids. The synthesized compounds were screened for **anticancer**, **antimicrobial**, and **anti-inflammatory** activities using standard in vitro assays. Results are presented in Table 2.

Table 2: Biological activity data of synthesized compounds.

Compound	IC50 (µM, HeLa) µg/ml	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	% Inhibition (Anti-inflammatory)
		E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*	*C. albicans*	
7a	-	----	----	----	27	13	-25.5
7b	-	10	----	----	39	21	62.9
7c	527.8	----	----	----	----	----	49.7
7d	716.26	----	----	----	14	----	29.4
7e	183.88	----	----	----	25	22	43.1
7f	63.99	----	----	----	30	17	4.3
7g	141.77	----	----	----	27	11	35.4
7h	550.2	----	----	----	----	----	44.5
7i	508.7	----	----	----	----	----	46.3
7j	87.54	----	----	----	28	14	2.6
Ampicillin (10 mcg)	----	----	----	10	34	26	----
Tetracycline (10 mcg)	----	20	----	14	29	19	----
Gentamicin (10 mcg)	----	28	20	24	22	16	----

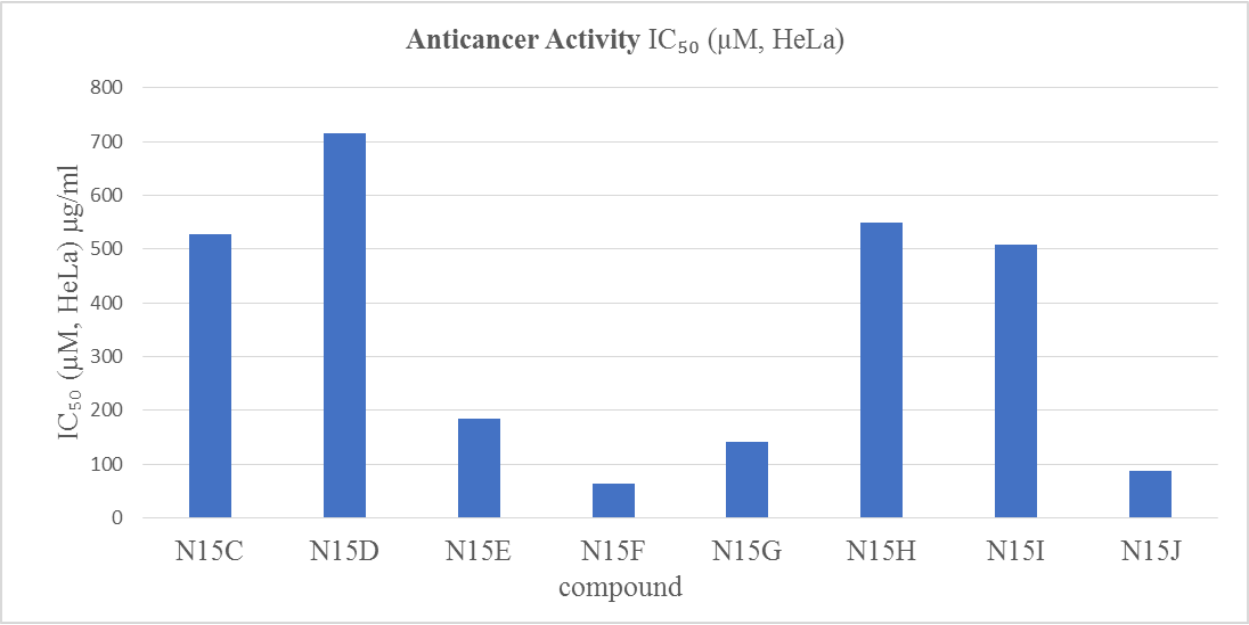


Fig. 1: In vitro anticancer activity of newly synthesized compounds.

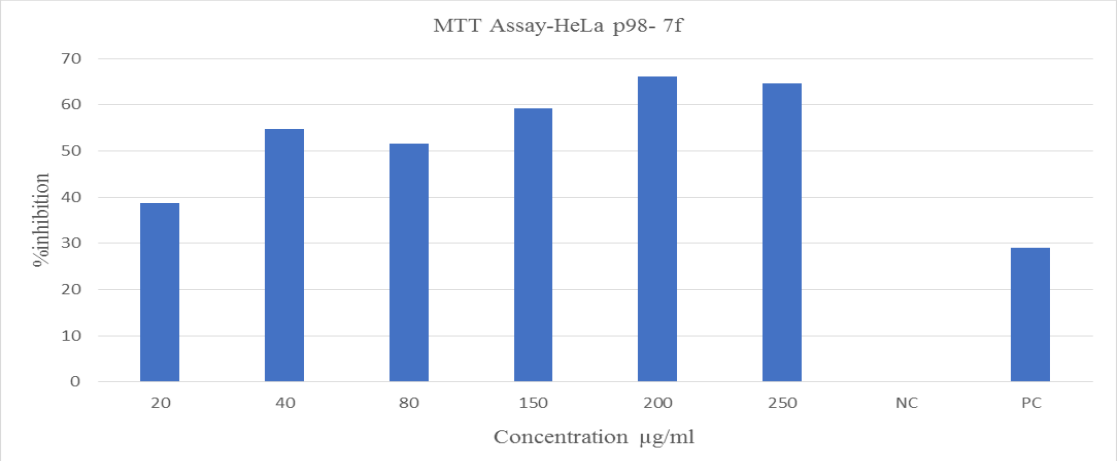


Fig. 2: In vitro anticancer activity against HeLa cell line for compound 7f with different concentration.

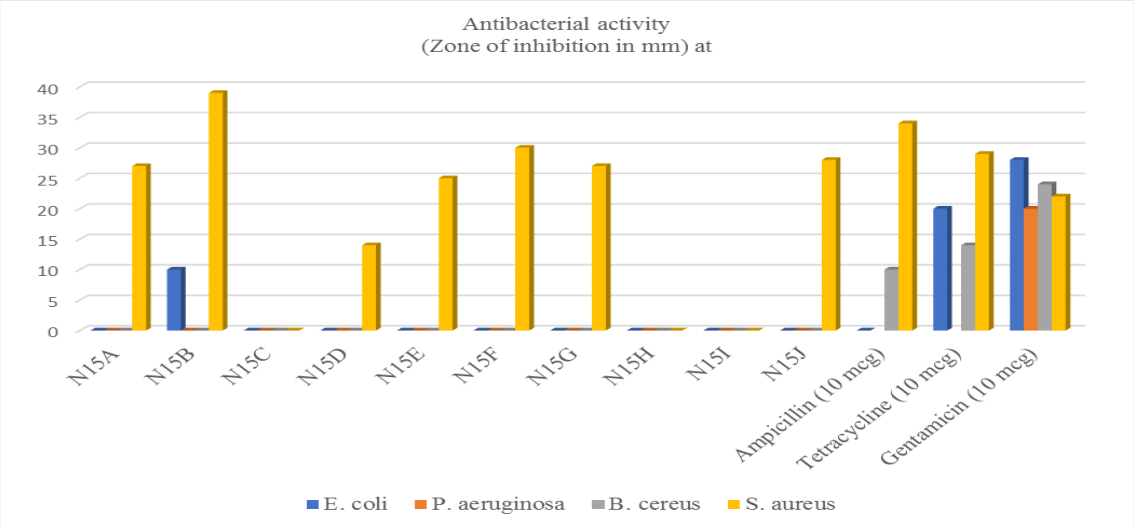


Fig. 3: In vitro antibacterial activity of newly synthesized compounds.

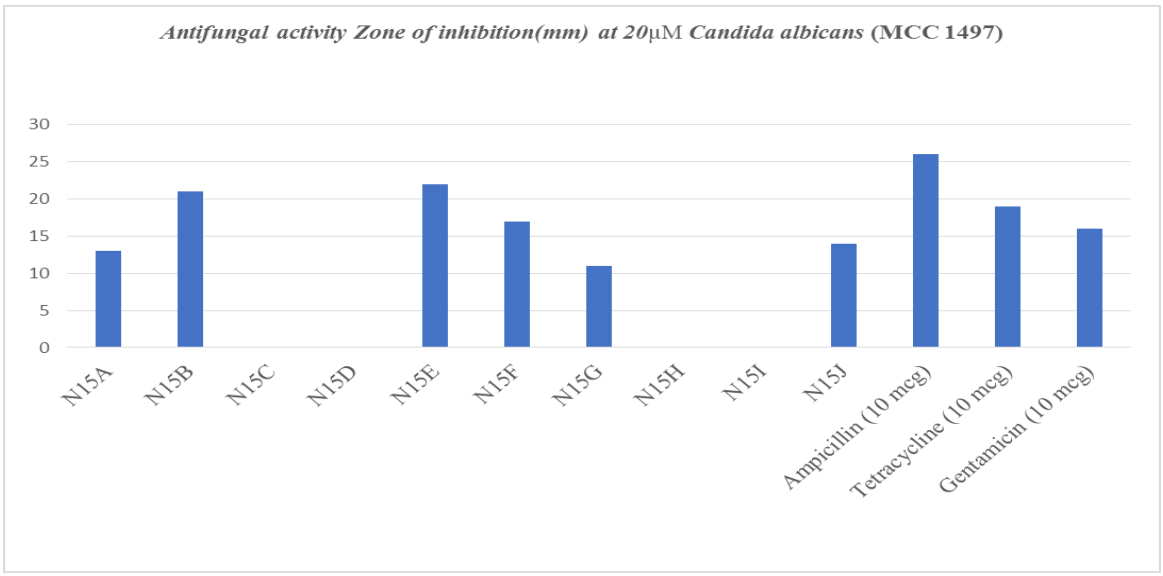


Fig. 4: In vitro antifungal activity of newly synthesized compounds.

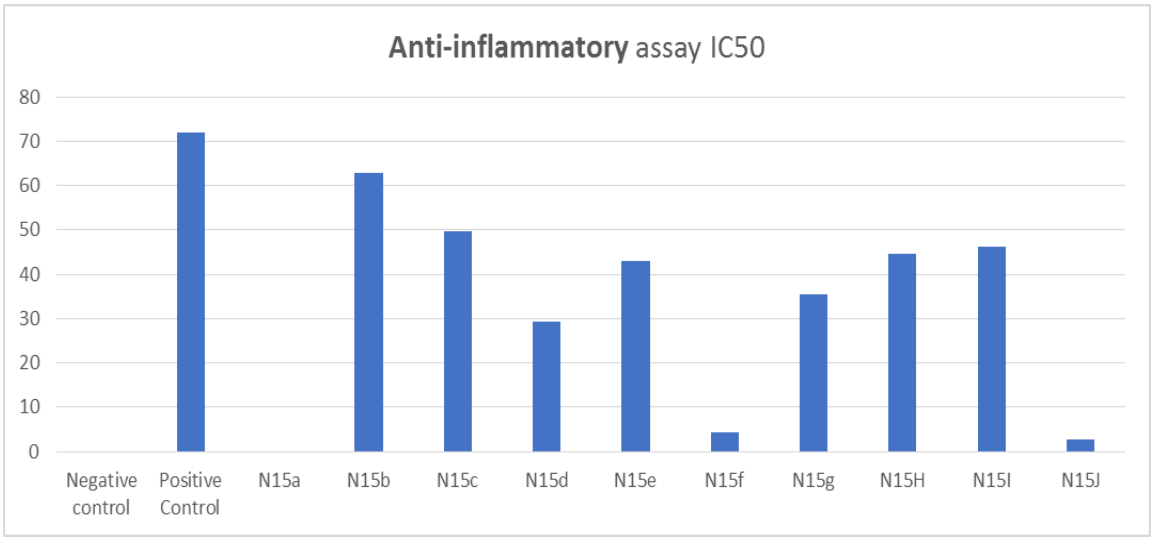


Fig. 5: In vitro Anti-inflammatory activity of newly synthesized compounds.

3. CONCLUSION

A novel series of 4-((substituted benzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol derivatives (7a-7j) was efficiently synthesized using a simple condensation route under mild conditions. Spectroscopic analyses confirmed the successful formation of Schiff base linkages and the integrity of the triazole–thiol framework. Biological investigations demonstrated that substitution on the benzylidene ring significantly modulated activity across all assays. Compounds bearing halogen substituents, particularly **7f (2-Cl)** and **7b (4-Cl)**, exhibited the most potent anticancer and antimicrobial activities, while **7b** also showed excellent anti-inflammatory properties, nearing the activity of the standard drug diclofenac. These results highlight the importance of electronic effects—especially electron-withdrawing groups—in enhancing bioactivity.

Overall, the study establishes the **1,2,4-triazole-3-thiol** core as a versatile pharmacophore capable of yielding multifunctional bioactive agents. The promising outcomes of chlorine-substituted analogues provide a strong basis for **further structural optimization**, **mechanistic studies**, and **in vivo evaluations** to advance the development of new therapeutic leads.

4. Experimental

4.1. materials and methods

All chemicals and solvents used in this study were of analytical grade and procured from Sigma-Aldrich, Merck, or HI Media (India). The starting material, **5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol**, was synthesized according to a previously reported procedure. Substituted aromatic aldehydes, absolute ethanol, and glacial acetic acid were used as received without further purification. All reactions were performed under standard laboratory conditions unless otherwise specified. The progress of reactions was monitored by **thin-layer chromatography (TLC)** on silica gel 60 F_{254} plates (Merck), and spots were visualized under ultraviolet light. **Melting points** were determined using the open capillary method and are uncorrected. **Infrared (IR) spectra** were recorded on a *Shimadzu FTIR spectrophotometer* using KBr pellets. **1H NMR** and **13C NMR spectra** were recorded on a *Bruker Avance 400 MHz spectrometer* using DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard. **Mass spectra** were obtained using an *electrospray ionization (ESI) mass spectrometer*. **Elemental analyses (C, H, N)** were carried out to confirm the purity and composition of the synthesized compounds.

4.2 preparation of oxadiazole

To a solution of isoniazide (1.0eq) in isopropanol : methanol(9:1) (10 eq) potassium ethyl xenthate (1.1eq) was added at room temperature and stirred for 10 minute, resulting mixture was heated at 80°c temperature for about 4 hour, completion the reaction was confirmed by TLC monitoring (using ethyl acetate : n-hexane (5:5) as mobile phase), reaction mixer was concentrated under reduce pressure to obtained yellow solid mass which was dissolve in water(5.0 vol) filter and follow by neutralization of filtrate using 50% HCL .resulting solid was filtered wash with cold water to remove access of acid and dried.

4.3 preparation of triazole

A solution of compound **2** (1.79 gram 0.01 mole) in n-butanol was refluxed with hydrazine hydrate (1.2 ml 0.25 mole) for 6 hour (control with TLC) after cooling the reaction mixture to room temperature the mixture was kept overnight in cold the resulting solid separated was collected by filtration and recrystallized to afford the Desire product **3** recrystallization to form ethanol to give cream solid

4.4. synthesis of 4-((substituted benzylidene)amino)-5-(pyridin-4-yl)-4h-1,2,4-triazole-3-thiol derivatives (7a)

A mixture of 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (0.01 mol) and the appropriate substituted benzaldehyde (0.01 mol) was dissolved in 25 mL of absolute ethanol.

To this, 3–4 drops of glacial acetic acid were added as a catalyst. The reaction mixture was refluxed for 4–6 hours with constant stirring. The completion of the reaction was monitored by TLC using ethyl acetate:n-hexane (7:3) as the mobile phase. After completion, the reaction mixture was cooled to room temperature, and the precipitated solid was filtered, washed with cold ethanol, and recrystallized from ethanol to obtain pure Schiff base derivatives 7a in good yield.
Other compounds(7b-7j) were synthesized following the same protocol as described above.

4.5. spectral characterization of final compounds

(E)-4-((2-nitrobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7a)
Yellow-orange solid; Yield 94 %; m. p. 214-216°C; IR (KBr, ν cm^{-1}): 3108 (N-H str.) 2849 (aromatic C–H), 1708 (C =N, str.); 1596 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.105 (s, 1H, SH), 9.012 (s, 1H, CH), 8.800-8.435(m, 4H, py), 8.182-7.018 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.65 (C=S), 149.25 (C=N), 143.85 (CH=N), 151.32-122.30 (aromatic carbons); ESI–MS: m/z calculated 326.00, found [M +H]+327.00

(E)-4-((4-nitrobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7b)
Yellow solid; Yield 95 %; m. p. 210-212 ^{o}C; IR (KBr, ν cm^{-1}): 3108 (N-H str.) 2849 (aromatic C–H), 1708 (C =N, str.); 1596 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.108 (s, 1H, SH), 8.819-8.806 (m, 2H, py), 8.502 (s, 1H, CH), 8.069-8.066 (m, 2H, py), 7.782-7.608 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.67 (C=S), 151.10 (C=N), 140.54 (CH=N), 151.32-120.06(aromatic carbons); ESI–MS: m/z calculated 326.00, found [M +H]+327.00

(E)-4-((4-chlorobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7c)
Light yellow solid; Yield 94 %; m. p. 202-204 ^{o}C; IR (KBr, ν cm^{-1}): 3116 (N-H str.) 2859 (aromatic C–H), 1710 (C =N, str.); 1616 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.108 (s, 1H, SH), 8.819-8.806 (m, 2H, py), 8.502 (s, 1H, CH), 8.069-8.066 (m, 2H, py), 7.782-7.608 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.37 (C=S), 150.76 (C=N), 150.58 (CH=N), 151.34-122.35(aromatic carbons); ESI–MS: m/z calculated 315.00, found [M +H]+316.10

(E)-4-((2-hydroxybenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7d) Cream solid; Yield 90 %; m. p. 190-192 ^{o}C; IR (KBr, ν cm^{-1}): 3208 (O-H str.), 3106 (N-H str.), 2849 (aromatic C–H), 1708 (C =N, str.); 1616 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.112 (s, 1H, SH), 8.502 (s, 1H, CH), 8.075-7.518(m, 4H, py), 7.298-6.900 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.63 (C=S), 159.25 (C-OH), 150.62 (CH=N), 151.33-120.06 (aromatic carbons); ESI–MS: m/z calculated 297.07, found [M +H]+298.10

(E)-4-((4-methoxybenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7e) Cream crystals; Yield 91 %; m. p. 182-184 ^{o}C; IR (KBr, ν cm^{-1}): 3110 (N-H str.), 2855 (aromatic C–H), 1715 (C =N, str.), 1598 (C=C str.) 1275 (C-O str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.108 (s, 1H, SH), 8.819-8.806 (m, 2H, py), 8.502 (s, 1H, CH), 8.069-8.066 (m, 2H, py), 7.782-7.608 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.66(C=S), 161.48 (C-OCH$_3$,149.20(C=N), 148.55(CH=N), 151.32-114.77 (aromatic carbons), 55.35(CH$_3$); ESI–MS: m/z calculated 311.08, found [M +H]+312.08

(E)-4-((3-bromobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7f) Yellow solid; Yield 92 %; m. p. 210-212 ^{o}C; IR (KBr, ν cm^{-1}): 3106 (N-H str.) 2847 (aromatic C–H), 1709 (C =N, str.); 1598 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.107 (s, 1H, SH), 8.750-8.742 (m, 2H, py), 8.485 (s, 1H, CH), 8.069-8.066 (m, 2H, py), 7.782-7.608 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.66(C=S), 150.50(CH=N), 149.20(C=N), 151.32-122.30(aromatic carbons); ESI–MS: m/z calculated 360.98, found [M +H]+361.98

(E)-4-((4-hydroxybenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7g) Cream solid; Yield 89 %; m. p. 185-187 ^{o}C; IR (KBr, ν cm^{-1}): 3208 (O-H str.), 3106 (N-H str.), 2849 (aromatic C–H), 1708 (C =N, str.); 1616 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.114 (s, 1H, SH), 8.751-8.746 (m, 2H, py), 8.545 (s, 1H, CH), 7.924-7.909 (m, 2H, py), 7.533-6.906 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.31 (C=S), 155.24 (C-OH), 140.54 (C=N), 131.65 (CH=N), 151.32-122.32(aromatic carbons); ESI–MS: m/z calculated 297.07, found [M +H]+298.10

(E)-4-((2-chlorobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7h) Pale orange solid; Yield 88 %; m. p. 208-210 ^{o}C; IR (KBr, ν cm^{-1}): 3116 (N-H str.) 2859 (aromatic C–H), 1710 (C =N, str.); 1616 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.105 (s, 1H, SH), 9.012 (s, 1H, CH), 8.800-8.435(m, 4H, py), 8.182-7.018 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.65(C=S),149.14(C=N),144.96(CH=N)151.32-122.30(aromatic carbons); ESI–MS: m/z calculated 315.09, found [M +H]+316.09

(E)-4-((4-methylbenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7i) Pale yellow solid; Yield 89 %; m. p. 188-190 ^{o}C; IR (KBr, ν cm^{-1}): 3106 (N-H str.) 2849 (aromatic C–H), 1706 (C =N, str.); 1594 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.112 (s, 1H, SH), 8.751-8.746 (m, 2H, py), 8.545 (s, 1H, CH), 7.924-7.909 (m, 2H, py), 7.533-6.906 (m, 4H, aromatic) 2.315 (s, 3H, CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.66(C=S), 149.20(CH=N), 148.83(C=N), 151.32-122.30(aromatic carbons), 21.41(CH$_3$); ESI–MS: m/z calculated 295.09, found [M +H]+296.09

(E)-4-((4-bromobenzylidene)amino)-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (7j) Yellow-orange solid; Yield 90 %; m. p. 206-208 ^{o}C; IR (KBr, ν cm^{-1}): 3106 (N-H str.) 2847 (aromatic C–H), 1709 (C =N, str.); 1598 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.108 (s, 1H, SH), 8.819-8.806 (m, 2H, py), 8.502 (s, 1H, CH), 8.069-8.066 (m, 2H, py), 7.782-7.608 (m, 4H, aromatic); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.65 (C=S), 149.25 (C=N), 145.83 (CH=N),151.32-122.30(aromatic carbons) ESI–MS: m/z calculated 360.98, found [M +H]+361.98

Credit Authorship Contribution Statement

Nilesh B. Patel: Writing – original draft. Faiyazalam M. Shaikh: Supervision. Navin B. Patel: Conceptualization. Vatsal M. Patel: Writing – review & editing, Supervision, Conceptualization.

Declaration Of Competing Interest

The authors declared no conflict of interest.

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REFERENCES

1. Patel, K. R.; et al. Review: Challenges of Chemotherapy and Emerging Small-Molecule Strategies in India. *Indian J. Pharmacol*, 2022; *54:* 101–116.
2. Reddy, K. R.; Rao, M. L.; Kumar, A. S. S. Drug resistance in cancer: Indian perspectives and small-molecule approaches. *Curr. Med. Chem*, 2023; *30*: 145–162.
3. Chauhan, H.; Agarwal, V.; Jain, R. ADMET challenges and resistance mechanisms: implications for Indian medicinal chemistry research. *J. Chem. Inf. Model*, 2023; *63*: 3211–3226.
4. Aggarwal, R.; Yadav, A.; Kar, S. An Insight on Medicinal Attributes of 1,2,4-Triazoles. *Front. Pharmacol*, 2020; *11:* 431.
5. Deb, M.; Banerjee, S.; Ghosh, S.; Roy, S. Di-Arylated 1,2,4-Triazole-Based Derivatives: Synthesis and Anticancer Activity. *J. Med. Chem*, 2024; *67:* 1145–1158.
6. Verma, P.; Kumar, D.; Singh, R. 1,2,4-Triazole Scaffold: Synthesis, Diversification and Biomedical Applications. *Chem. Biol. Drug Des*, 2024; *103:* 145–166.
7. Raut, S.; Shinde, S.; Kulkarni, P. Triazole-Containing Compounds as Anticancer Agents: Synthesis and Biological Perspective. *Curr. Top. Med. Chem*, 2024; *24:* 191–213.
8. Khan, J.; et al. Triazole-Containing Drugs: Clinical Approvals and Therapeutic Classes — an Indian review. *Eur. J. Med. Chem*, 2024; *260:* 114421.
9. Nawaz, Z.; et al. Design, Synthesis, and DFT Studies of Triazole Derivatives: Relevance to Clinical Triazoles. *Bioorg. Chem*, 2024; *125*: 105885.
10. Kulkarni, R.; More, A.; Dumbre, R. Docking and In Vitro Anticancer Studies of Novel Triazole Schiff Base Ligands and Their Metal Complexes. *J. Biomol. Struct. Dyn*, 2024; *42:* 4043–4057.
11. Banerjee, S.; Mukherjee, S.; Ghosh, S. Triazole-Linked Schiff Bases as Promising Anticancer Agents: Synthesis, SAR and Mechanistic Studies. *Eur. J. Pharm. Sci*, 2023; *170*: 106071.
12. Vernekar, M.; Deshmukh, S.; Gaikwad, V. Lead Optimization of Triazole Derivatives Guided by SAR and Docking Studies: Anticancer Hits. *Eur. J. Pharmacol*, 2024; *929:* 175156.
13. Nambiar, A.; Koshy, N.; Sunny, P. Triazole Schiff Base Complexes of Cu(II) and Zn(II): Antimicrobial and Cytotoxic Studies. *J. Inorg. Organomet. Polym*, 2024; *34*: 2179–2194.
14. Siddiqui, A. A.; Rajasekaran, S.; Rajesh, R. Synthesis, Analgesic and Anti-Inflammatory Activities of Some Novel 5-Phenyl-4-Substituted-3-Mercapto(4H)-1,2,4-Triazoles. *Orient. J. Chem*, 2005; *21:* 365– 370.
15. Gupta, D.; Synthesis, Antifungal and Antibacterial Activity of Novel 1,2,4-Triazole-3-thiol Derivatives. *J. Heterocycl. Chem*, 2015; *52:* 345–356.
16. Sabale, P. M.; Mehta, P.; Prafulla. Synthesis and Antimicrobial Activity of Substituted 1,2,4-Triazole-3-thiols. *Der Pharma Chemica*, 2013; *5*: 149–154.
17. Research Group (compendium). S-Substituted Derivatives of 1,2,4-Triazol-3-thiol: syntheses and antimicrobial screening. *Indian J. Chem*, 2021; *60B*: 1002–1011.
18. Bajroliya, S.; Kalwania, G. S.; Choudhary, S.; Chomal, S. Synthesis, Characterization and Antimicrobial Activities of 1,2,4-Triazole/Isatin Schiff Bases and Their Mn(II), Co(II) Complexes. Orient. J. Chem, 2014; 30: 2419–2425.
19. Deodware, S. A.; Patil, R. R.; In Vitro Anticancer Screening, Molecular Docking and Biological Evaluation of Schiff-Base-Derived Metal Complexes. *Molecules.*, 2022; *27:* 7899.
20. Joshi, R.; Mehta, S.; Patel, K. S. Synthesis, Characterization and Antibacterial Studies of New 1,2,4- Triazole Schiff Bases. *Indian J. Chem., Sect. B.*, 2021; *60B*: 1002–1011.
21. Siddiqui, A. A.; Synthesis of 4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole derivatives and related Schiff bases. *Acta Pharmaceutica*/J. Indian Org. Chem, 2010; *?*, 1–12.
22. Oderinlo, O. O.; Hydrazone-Tethered 5-(Pyridin-4-yl)-4H-1,2,4-triazole-3-thiol Hybrids: Synthesis, ADME and in vitro activities (collaboration with Indian groups). *ChemMedChem*, 2023; *18:* 2000–2010.
23. Elumalai, P.; Karthikeyan, M.; Synthesis, Docking and Biological Evaluation of 1,2,4-Triazole-Derived

Schiff Bases as Multifunctional Agents. *J. Mol. Struct*, 2024; *1302:* 134672.
24. Yadav, P.; Suresh, R.; Kumar, S. Structure–Activity Relationship Study of Triazole-Based Schiff Bases: Influence of Substituent Electronics. *ACS Omega*, 2024; *9:* 15802–15815.
25. Prasad, A.; Yadav, R.; Singh, A. Design and Biological Evaluation of Halogenated Triazole Schiff Bases: Correlation of Lipophilicity with Activity. *ChemMedChem*, 2022; *17*: e202200234.
26. Siwach, P.; Mehta, R.; Synthesis, Characterization and Antimicrobial Activity of 1,2,4-Triazole Derivatives: SAR Study. *Appl. Organomet. Chem*, 2025; *39*: e70117.
27. Rao, D. P.; Kumar, S.; Schiff Bases and Their Therapeutic Applications: Recent Trends and Future Perspectives. *Eur. J. Med. Chem*, 2024; *266*: 115112.
28. Mohile, M.; Gajera, B.; Solanki, P. Synthesis and Evaluation of Anticancer Activity of Novel Triazole Derivatives Against HeLa and MCF-7 Cells. *Pharm. Chem. J.*, 2023; *57*: 256–266.
29. Mannaa, A. H.; El-Sayed, R.; Bivalent Transition Metal Complexes of Triazole–Pyridine Schiff Base Ligands: Synthesis and Biological Activities (Indian collaborations). *Sci. Rep*, 2025; *15*: 15782.
30. Chauhan, S.; Kaur, R.; Arora, S. Hybrid Triazole–Thiadiazole Derivatives: Synthesis and Biological Profiling. *Tetrahedron Lett*, 2021; *76:* 153258.
31. Patel, N. B.; Soni, H. I.; Parmar, R. B.; Chan-Bacab, M. J.; Rivera, G., 1, 2, 4-triazoles clubbed pyrimidine compounds with synthesis, antimicrobial, antituberculosis, antimalarial, and anti-protozoal studies. *Letters in Organic Chemistry*, 2021; *18*(8): 617-624.
32. Ameen, D. S. M.; Hamdi, M. D.; Khan, A. K., Synthesis and biological activities of some 1, 2, 4-triazole derivatives: A review. *Al Mustansiriyah Journal of Pharmaceutical Sciences*, 2022; *22*(3): 65-81.
33. Karpun, Y.; Polishchuk, N., Synthesis and antimicrobial activity of s-substituted derivatives of 1, 2, 4-triazol-3-thiol, 2021.
34. Singh, G.; Malik, S.; Gupta, S.; Kaur, H.; Dalal, A.; Khurana, S.; Kaur, A., Synthesis, structural characterization, and potential antifungal activity of schiff base probes: A molecular docking study. *Journal of Molecular Structure* 2025; *1337:* 142136.
35. Zheng, A. L. T.; Boonyuen, S.; Li, G. Y.; Ngee, L. H.; Andou, Y., Design of reduced graphene hydrogel with alkylamine surface functionalization through immersion/agitation method and its adsorption mechanism. *Journal of Molecular Structure* 2021, *1245*, 131008.
36. Aitha, S.; Shankar, B.; Matta, R.; Srinivas, M.; Pochampally, J., Synthesis and Evaluation of Molecular Docking and Anti-Inflammatory Activity of New 1, 2, 3-Triazole Derivatives. *Journal of Chemistry*, 2026; *2026*(1): 9411347.
37. Saravanan, G.; Pannerselvam, P.; Prakash, C. R., Synthesis and anti-microbial screening of novel Schiff bases of 3-amino-2-methyl quinazolin 4-(3H)-one. *Journal of Advanced Pharmaceutical Technology & Research*, 2010; *1*(3): 320-325.
38. Sabale, P. M.; Mehta, P., SYNTHESIS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NOVEL 4-AMINO-SUBSTITUTED-1, 2, 4-TRIAZOLE-3-THIOL DERIVATIVES. *Indian Journal of Heterocyclic Chemistry*, 2013; *23*(2): 149-154.