Designed synthesis and invitro evaluation of (E)-8,8-dimethyl-5-phenyl-2-styryl-5,7,8,9-tetrahydro-6H-[1,3,4] thiadiazolo[2,3-b]quinazolin-6-one promoted by Copper acetate

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ABSTRACT:

In this paper, a simple and an efficient protocol was followed and the synthesis of novel substituted 8,8-dimethyl-5-phenyl-2-styryl-5,7,8,9-tetrahydro-6H-[1,3,4]thiadiazolo [2,3-b]quinazolin-6-one(5a-5i). The title compounds were synthesized by the reaction of 5-styryl-1, 3, 4-thiadiazol-2-amine**,** substituted benzaldehyde and dimedone promoted by transition metal acetates in ethanol. The compound (3) can be prepared by cinnamic acid with thiosemicarbazide in con H_2SO_4 in Toluene as solvent. The structures of all the newly synthesized derivatives have been interpreted on the basis of analytical and spectral data viz ^{1}HNMR, ^{13}CNMR and LCMS. This method's many advantages include a large yield, a short reaction time, mild reaction conditions, ease of operation, an environmentally benign work-up procedure, and the purification of products using non-chromatographic techniques. The anti-microbial activity was screening demonstrated that majority of the identified compounds were found to have significant anti-microbial activity activities The produced substances' antibacterial efficacy against a range of microbiological strains at varying doses was assessed.

KEYWORDS:

Thiadiazolo [2, 3-b] quinazolin-6-ones, cinnamic acid, thiosemicarbazide, aromatic aldehyde, dimedone, copper acetate, bioevluation

INTRODUCTION:

They contain one or more heteroatoms. The most prevalent ones are tetragon, pentagonal, hexagonal, and triple, and they are made of nitrogen, oxygen, and sulfur. Azoles, another name for thiophene, can include two distinct atoms. Due to its diverse coordination capacity towards mineral element ions, the nitrogen and sulphur thiadiazolo ring has gained a lot of attention, notably in structures, biological applications, and bioactive compounds. The isomer 1, 3, 4 thiadiazole takes on a much larger relevance when compared to the other three isomers. In this field of modern chemistry, the identification, isolation, and purification of active compounds from plant, microbe tissues as well as from their fermentation products has generated interest and drawn attention from researchers across the globe

A bacterial infection remains one of the top causes of death despite tremendous advancements in our understanding of its pathophysiology. Therefore, the discovery of new, more potent bacterial infection treatments is desperately needed. Small compounds with five-member heterocyclic moieties have drawn a lot of attention in recent years as potential novel bacterial infection drugs. One such is the 1, 3, 4-thiadiazole, a flexible scaffold that is extensively researched in medicinal chemistry.

Many chemicals with biological action have heterocyclic moieties. The chemicals' molecular structures mostly determine their biological function [1].1, 3, and 4-thiadiazoles

are highly intriguing substances because of their significant uses in a variety of biological, analytical, and pharmacological fields [2, 3]. Because of their substantial and adaptable biological activity [4], sulfur-nitrogen heterocycles are key molecules that have maintained researchers' interest. One of the most promising classes of heterocycles in drug discovery is represented by 1, 3, and 4-thiadiazoles. Commercially accessible 1, 3, 4-thiadiazole medications include furidiazine, desaglybuzole, and acetazolamide [5].The presence of the –N=C=S moiety [7] may be the cause of the thiadiazole nucleus's many biological activity [6]. Many classes of thiadiazole compounds have been the subject of extensive research in recent years. These compounds are known to have intriguing biological properties, including Biological evaluation [1-4], Antimicrobial activity [5-11], anticancer agents [12,13], Herbicidal activity [14], antitumor activity [15], antiproliferative activity [16], antiviral activity [17] .The previous literature reveals that the designed derivatives were synthesized by employing the various catalyst such as Cyanuric Chloride [18], Transition Metal [19], diphenhydramine hydrochloride [20]

Therefore, in this research, our on-going to work was stage wise the reaction procedure by the use of a dehydrating reagent system and analogous of target compounds substituted (E)-8,8-dimethyl-5-phenyl-2-styryl-5,7,8,9-tetrahydro-6H-[1,3,4]thiadiazolo [2,3-b]quinazolin-6-ones were obtained by two steps. These target compound can be obtained by (E)-5-styryl-1, 3, 4-thiadiazol-2-amine**,** substituted benzaldehyde and dimedone promoted by transition metal acetates in ethanol. The compound (3) can be prepared by cinnamic acid with thiosemicarbazide in con H_2SO_4 in Toluene as solvent

2. MATERIAL AND METHODS:

2.1. Experimental

With the following exclusions, the starting components, such as reagents and solvents, were purchased from SRL and Merck, India Aldrich chemicals, and were utilised without before being purified. Calcium hydride was distilled under nitrogen to produce methanol and dimethyl formamide. Using silica gel 60 (Merck) and the specified solvents, flash column chromatography was carried out. Kiesel gel F254 plates (Merck) were used for thin-layer chromatography (TLC). The Toxi Spin (400MHz & 100MHz) spectrometer was used to record the 1H and ^{13}CNMR spectra of solutions in either deuteriodimethyl sulphoxide $(CD_3)_2SO$) or deuteriochloroform ($CDCl_3$). Chemical deviations from an internal standard, tetramethyl silane, are reported in parts per million (ppm) downfield

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2.2. Preparation of (E)-5-styryl-1,3,4-thiadiazol-2-amine (3):

Four RBF necks should be cleaned and dried. At room temperature, the mixture of 1 mol of cinnamic acid (1mole) and semithiocarbazide (1mole) was dissolved in 25 mL of toluene and a few drops of conc H_2SO_4 in RBF, which was also attached to a magnetic stirrer with a hot plate. For five hours at 100^0C, the reaction was continuously carried out by the reaction mixture. The TLC was used to monitored the reaction's development (EtOAc: n-hexane = 5:5) and the reaction mixture was cooled at room temperature. The crude was extracted in ethyl acetate and this layer was separated by washing, it with a saturated sodium bicarbonate solution. The organic layer was separated by washing it with water as well and subsequently followed by vacuum and the organic layer can be removed by distillation, yielding a solid compound

White solid; yield-95%, m.p-171-173^{0}C: ^{1}HNMR (400 MHz, $CDCl_3$) δppm: 11.541 (N-H, Pyrrole, s), 8.425 (pyrole,s), 7.754 (1H,s), 7.394-7.358 (Ar,m,2H), 6./87 (2H, NH_2,s). ^{13}CNMR (400MHz, $CDCl_3$) δppm: 173.58, 161.33, 142.58, 136.75, 128.87, 127.39, 124.54, 121.94, 114.02, 111.40; Molecular weight (m/z): 496.27(M+2); Formulae of compound: $C_{10}H_9N_3S$. Elemental Analysis: Calculated; C-72.61, H-5.61,N- 10.16: Obtained: C-72.55 ,H-5.60,N- 10.24

2.3. General procedure for the synthesis of (E)-8,8-dimethyl-5-phenyl-2-styryl-5,7,8,9-tetrahydro-6H-[1,3,4]thiadiazolo[2,3-b]quinazolin-6-one

The mixture of 5-styryl-1,3,4-thiadiazol-2-amine1(1mol), aromatic aldehydes **2** (1mol), dimedone (**3)** (1 mol) and are dissolved in ethanol in 25mL RBF and transition metal acetate such as copper acetate(10mmole) charged into above solution. The reaction was continued at 70°C for 7hrs time to complete the reaction and it was monitored by TLC (4:6 = EtOAc: n-hexane) and then 50mL water was added after completion of the reaction. The solid was filtered off and extracted with Ethylacetae and washed with Braine. The crude was purified by recrystallization from absolute to afford the product

2.3.1. COMPOUND-6a

Pale-yellow, M.P-231-233^{0}C; Yield-84%, 1HNMR (400MHz, $CDCl_3$)δppm: 7.617-7.394 (m,5H,Ar), 7.3627.271 (m,4H,Ar), 6.557(d, J=15.8Hz, 1H), 6.367 (d, J=15.8Hz, 2H), 4.294(s, 1H, C4), 2.040 (s, 1H, C-4), 1.559 (s, 1H), 1.046 (s, 6H(CH3)2); 13 CNMR 100 MHz, CDCl3) δppm: 190.02(2C), 161.71 (1C), 155.02 (1C), 151.76 (1C), 137.74 (1C), 135.02 (1C), 131.67 (1C), 129.44 (1C), 128.86 (2C), 128.49 (2C), 128.14 (2C), 127.76 (2C), 127.15 (1C), 126.68 (1C), 120.86 (1C), 62.71 (1C), 47.46 (1C), 36.62(1C), 31.74(1C), 29.44(1C), 26.61(1C) ; Molecular Formula of the compound : $C_{25}H_{23}N_3O_5$; Molecular weight of the compound: 414.38(M+H); Elemental Analysis: Calculated; C- 72.61,H-5.61,N- 10.16 : Obtained: C-72.54,H5.59,N-10.24

2.3.2. COMPOUND-6b

Pale-yellow, M.P-228-230^{0}C; Yield-94%, 1HNMR (400MHz, CDCl3) δppm: 9.219 (s, 1H, OH), 7.526-7.336 (m, 5H, Ar), 7.128-6.896 (m, 4H, Ar), 6.619 (d, J=12.5Hz, 1H), 6.284 (d, J=12.5Hz, 1H), 4.326 (s, 1H, C-4), 2.062 (s, 2H, CH2), 1.552 (s, 2H, CH2), 0.984 (s, 6H, (CH3)2),^{13}CNMR(100MHz, $CDCl_3$)δppm: 192.06 (1C), 160.02 (1C), 156.72 (1C), 149.44 (1C), 145.78 (1C), 137.71 (1C), 133.06 (1C), 131.74 (1C), 129.49 (1C), 128.75 (2C), 128.19 (2C), 127.44 (1C), 127.02 (2C), 125.79 (2C), 122.86 (1C), 65.62 (1C), 49.14 (1C), 41.02 (1C), 30.28(1C),29.01(1C), 27.24(1C);Molecular Formula of the compound : $C_{25}H_{23}N_3O_2S$; Molecular weight of the compound is: 429.41(M+), 430.76(M+H) ;Elemental Analysis : Calculated; C- 69.91, H- 5.40,N-9.78: Obtained: C-69.85 ,H-5.38, ,N-9.85

2.3.3. COMPOUND-6c

Pale-yellow.- M.P-252-253^{0}C; Yield-90%, 1HNMR (400MHz, CDCl3)δppm: 7.716-7.306 (m,5H,Ar), 7.198-7.021 (m,4H,Ar), 6.508(d, J=12.9Hz, 1H), 6.236 (d,J=12.9Hz, 1H), 4.376 (s,1H,C-4), 3.726 (s,3H,OCH3), 1.962 (s,2H,CH2), 1.556 (s,2H,CH2), 1.046 (s,3H,CH3), 0.973 (s,3H,CH3) ;13CNMR (100MHz,CDCl3)δppm: 194.62 (1C), 159.42 (1C), 154.02 (1C), 149.17 (1C), 139.04 (1C), 134.19(1C), 132.46(1C), 130.66(1C), 129.47(2C), 128.88(2C), 127.16(1C), 126.08(2C), 123.76(2C), 120.98(1C), 62.76(1C), 48.77(1C), 37.62(1C), 30.62(1C), 28.06(2C); Molecular Formula of the compound : $C_{26}H_{25}N_3O_2S$;

Molecular weight of the compound is: 442.61(M-H), 443.19(M+),444.88(M+H) ;Elemental Analysis : Calculated; C- 70.40, H- 5.68,N-9.47: Obtained: C- 70.34 ,H-5.66, N- 9.55

2.3.4. COMPOUND-6d:

Paleyellow,224-226^{0}C;Yield-91%, 1HNMR(400MHz,$CDCl_3$)δppm: 7.574-7.287 (m, 5H,Ar), 7.256-7.021 (m,4H,Ar), 6.724 (d, J=15.6Hz, 1H), 6.584 (d, J=15.6Hz, 1H), 4.816 (s, 1H, C-4), 2.119 (s, 2H, CH_2), 1.714 (s, 2H, CH2), 1.046 (s, 6H, $(CH_3)_2$) ; 13CNMR(100MHz, $CDCl_3$)δppm: 197.66 (1C),162.74(1C), 153.06 (1C), 139.62 (1C), 137.74 (1C), 134.06 (1C), 131.65(1C),129.64 (1C), 129.15 (2C), 128.79(2C), 128.44(2C), 127.65(1C), 127.04(2C), 121.62(1C), 63.76 (1C) ,51.15 (1C), 38.62 (1C), 32.02 (1C), 29.06 (1C), 27.62(2C); Molecular Formula of the compound is: $C_{25}H_{22}ClN_3OS$; Molecular weight of the compound is: 447.38(M+), 448.04(M+H),449.66(M+2) ;Elemental Analysis : Calculated; C- 67.03, H-4.95,N-9.38: Obtained: C- 66 . 98, H-4.94, N- 9.45

2.3.5. COMPOUND-6e:

Paleyellow,246-248^{0}C;Yield-89%,^{1}HNMR(400MHz, CDCl3) δppm : 7.846 (d, J=8.8Hz, 2H), 7.583 (d, J=6.4Hz, 2H), 7.486 -7.271 (m, 5H, Ar), 6.665 (d, J=14.5Hz, 1H), 6.446 (d, J=14.5Hz, 1H), 4.707(s, 1H, C-4) ,2.176 (s,2H,CH2) , 1.768 (s, 2H, CH2), 1.093 (s, 6H, (CH3)2); 13CNMR (100MHz, CDCl3)δppm: 198.49(1C), 163.21(1C), 157.74(1C), 153.66(1C), 139.52(1C), 136.74(1C), 134.71(1C), 130.64(1C), 129.46(1C), 129.02(1C), 128.79(2C), 128.33(2C), 127.46(1C), 127.06(1C), 120.84(1C), 66.67(1C), 51.84(1C), 39.64(1C), 30.62(1C), 28.48(1C), 27.15(1C); Molecular Formula of the compound : $C_{25}H_{22}BrN_3OS$;Molecularweight of the compound : 491.06 (M+), 492.69 (M+H), 493.25 (M+2) ;Elemental Analysis : Calculated; C- 60.98, H- 4.50,N-8.53: Obtained: C- 60.90, H-4.48, N- 8.65

2.3.6. COMPOUND-(6f):

Pale-yellow, M.P-239-241^{0}C ;Yield-88%, 1HNMR (400MHz, $CDCl_3$) δppm: 7.806-7.596 (m,4H,Ar), 7.504-7.284 (m,5H,Ar), 6.643(d,J=12.5Hz,1H), 6.146(d, J=12.5Hz, 1H), 4.491 (s,1H,C-4), 2.116 (s,2H,CH2), 1.554 (s,2H,CH2), .957(s,6H,(CH3)2) ;13CNMR (100MHz, CDCl3) δppm: 197.14 (1C), 160.62(1C), 155.06(1C), 150.87(1C), 141.07(1C), 137.36(1C), 134.65(1C), 129.65(1C), 129.02(2C), 128.81(2C), 128.22(2C), 127.96(1C), 127.28(2C), 124.71(1C), 119.09(1C), 117.66(1C), 66.67(1C), 48.27(1C), 39.18(1C), 30.26(1C), 28.15(1C), 26.79(1C); Molecular Formula of the compound :$C_{26}H_{22}N_4OS$; Molecular weight of the compound: 438.79(M+), 439.65(M+H), ;Elemental Analysis : Calculated; C- 71.21, H-5.06,N-12.76: Obtained: C- 71.15 , H-5.04,N-12.84

2.3.7. COMPOUND-(6g):

Pale-yellow, M.P- 245-247^{0}C; Yield-87%, ^{1}HNMR (400MHz, $CDCl_3$) δppm :8.076 (d, J=8.8Hz, 2H, Ar), .884 (d, J=8.0Hz, 2H), 7.549-7.294 (m, 5H, Ar), 6.881 (d, J=13.5Hz ,1H, CH=CH), 6.546 (d, J= 13.5Hz, 1H, CH=CH), 4.607 (s,1H,C4), 2.069 (s, 2H ,CH2), 1.613 (s, 2H, CH2), 1.049 (s ,6H, $(CH_3)_2$); 13 CNMR (100MHz ,CDCl3)δ ppm: 195.79 (1C), 164.02 (1C), 158.36 (1C), 150.49 (1C), 140.06 (1C), 138.24 (1C), 136.92 (1C), 132.38 (1C), 129.44 (1C), 128.91 (2C), 128.50 (2C), 127.92 (2C), 127.65 (1C), 127.33 (2C), 63.04 (1C), 52.74 (1C), 40.06 (1C), 30.47 (1C), 28.46 (2C); Molecular Formula of the compound :$C_{25}H_{22}N_4O_3S$;Molecular weight of the compound : 447.27(M-H), 448.14(M+),449.51(M+H)

;Elemental Analysis : Calculated; C- 65.59, H- 4.84,N-12.22: Obtained: C-65.51,H-4.82, ,N- 12.35

3. BIOLOGICAL EVALUATION:

3.1.1. Antimicrobial activity

All the prepared analogous were examined for *invitro* anti-microbial activities by paper disc diffusion technique. The anti-bacterial potent activity of the prepared derivatives was tested against strains isolated from animal byproducts and were accused of being a direct cause of food intoxication in human. The strains include three Gram (+Ve) -positive *bacteria S. aureus, and Bacillus cereu*s and three Gram (-Ve) bacteria E. coli and P. aeruginosa using Muller Hinton agar medium . The anti-fungal activities of the derivatives were tested against two fungi C. albicans and A. flavus applied by Sabouraud dextrose agar medium. The identification data on the anti-microbial activity of the synthesized compounds and standard drugs are given in Table 4:

Anti-bacterial activity

The tested derivatives were evaluated at concentrations of 250 µg/mL and 500 µg/mL applying DMSO as a solvent, the 10 µg/mL disc, and Streptomycin as a standard. The rest of the derivatives were recognised to be moderately active against the tested microorganism. The *invitro* anti-bacterial activities of the desired synthesized derivatives are evaluated against four pathogenic bacteria strains. The gram(+Ve) bacteria were examined *S. aureus and P. aeruginosa.*

Antifungal assay:

50 millilitres of a fungal spore suspension were aseptically added to sterile molten potato dextrose agar (PDA) medium, which was then kept at 45^0C. After thoroughly mixing the inoculated medium, it was quickly transferred into sterile Petri plates. Next, a sterile borer was used to punch five 6 mm diameter wells, which were then filled with 100 µlg/mL of the test compounds (6a–l) and 100% sterile DMSO as a negative control. The plates were incubated at 37^0C for a full day. By calculating the zone of inhibition, *invitro* antifungal activity was ascertained. The test compounds' zones were contrasted with those of "ketoconazole."

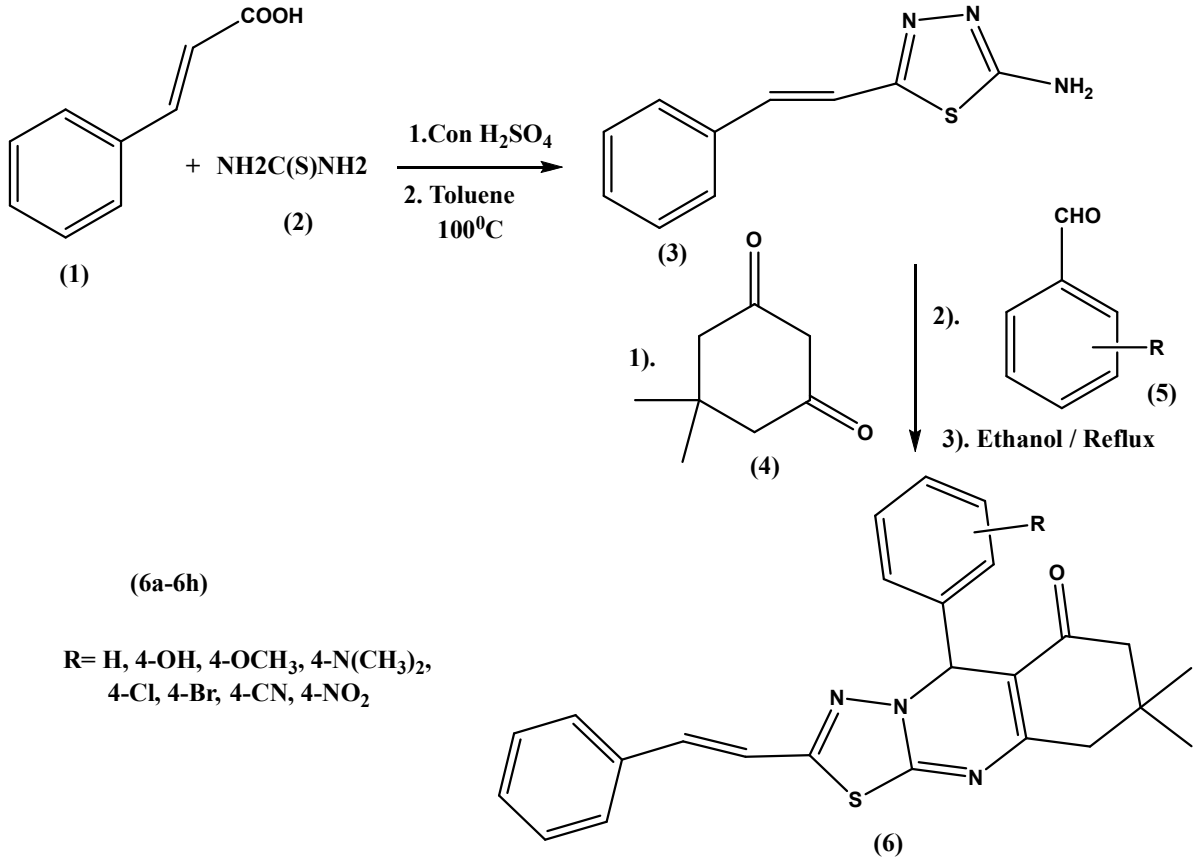
3.1.2. Antioxidant activity:

The solution was protected from light by covering the test tubes with aluminium foil. DPPH (4 mg) was dissolved in 100 mL of ethanol. Some of the produced compounds were used to make various concentrations of 25, 50, 100 ppm. It was made by dissolving 1 mg of the chemical in 10 mL of ethanol to make 100 ppm, then diluting it to 50 and 25 ppm. The concentrations were made in the same way. 1 mL of the diluted or normal solution 25, 50, 100 ppm was added to 1 mL of DPPH solution in a test tube. After 1 h of incubation at 37 °C, the absorbance of each solution was measured using a spectrophotometer at 517 nm. The following equation was used to determine the potential to scavenge DPPH radicals.

I% = (Absorption blank–Absorption sample)/Absorption blank × 100

4. RESULTS AND DISCUSSION:

4.1. Chemistry



(Scheme-1)

The synthesis of novel substituted (E)-8,8-dimethyl-5-phenyl-2-styryl-5,7,8,9-tetrahydro-6H-[1,3,4]thiadiazolo [2,3-b]quinazolin-6-one(5a-5i). The title compounds were synthesized by the reaction of (E)-5-styryl-1, 3, 4-thiadiazol-2-amine, substituted benzaldehyde and dimedone promoted by transition metal acetates in ethanol. The compound (3) can be prepared by cinnamic acid with thiosemicarbazide in con H_2SO_4 in Toluene as solvent

Initially, the preliminary identification this method of process and optimization of catalyst and the reaction conditions, the reaction was continued in absence of catalyst under solvent condition but desired product was not obtained even the reaction was time extending. So, the advantages and scope of catalyst is an essential for enhancement of the progress of reaction. An importance of catalyst, we maintained mild, an inexpensive, nontoxic, highly stable and readily available transition metal catalyst. The various types of transition metal acetate catalyst were applied in presence of various solvent solvents at different temperature during the reaction. After completion of the reaction, it was observed that the major product was obtained by Cu $(OAc)_2$ as catalyst as indicates Table-1

Table -1. Optimization of catalyst for synthesis of derivative (6b)

Entry	catalyst	Time (hrs)	Yields (%)
1	$Cu(OAc)_2$	7	92
	$Zn(OAc)_2$	7	70
3	$Mg(OAc)_2$	7	51
4	$Mn(OAc)_2$	7	63

Reaction of of thiazolidine-2, 4-dione, aromaticldehydes, benzyl cyanide and ammonium chloride in presence of $Cu(OAc)_2$ under conventional and solvent conditions, b; Isolated yield

Its exact measurement and observation, initially recognised the reaction that impact of the different aromatic aldehydes, compound (3), and dimedone in promoted by $Cu(OAc)_2$ as a catalyst under different circumstances in order to enhance the reaction. It was observed that the standard approach was applied (Scheme-1) and substituted aromatic aldehyde (1.15 mol), Ethylacatoacetate (1.25 mol) and compound (2), and $Cu(OAc)_2$ (4 mol %), good to outstanding results were acquired. The decreasing catalyst's quantity, it can result in a lower yield of the desired product. The equivalent product was scaffold in absence catalyst when the identical reaction was established without the catalyst. Under any circumstances, a number of substituted aromatic aldehydes were assessed; the outcomes are displayed in - Table 2.

Table-2 . Effect of amount of catalyst on derivative (6b)[a]

Entry	Catalysts (mmole)	Time (hrs)	Yield (%)[b*]
1	0	No	No
2	1	7	51
3	3	7	58
4	4	7	94
5	5	7	94

a: Reaction of of thiazolidine-2, 4-dione, aromaticldehydes, benzyl cyanide and ammonium chloride in presence of $Cu(OAc)_2$ under conventional and solvent conditions, b; Isolated yield

In additionally, we observed that the catalyst's impact on various solvents during the $Cu(OAc)_2$ based synthesis of designed derivatives is described in Table-3. The titled analogous were obtained by applied variety of solvents, including ethanol, water, DMF, MDC, and Toluene. We found that ethanol impact of work in reaction better as a solvent when synthesizing desired derivatives effected of catalyst as given table-3

Table -3: Optimization of solvent effect on the model reaction (6b):

Entry	Solvent	Time[a] (hrs)	Yield[b] (%)
1	Water	7	20
2	DMF	7	45
3	MDC	7	57
4	Toluene	7	63
5	Ethanol	7	92

a:Reaction compound (3), arylaldhyde, dimedone in promoted by $Cu(OAc)_2$ under conventional and solvent conditions, b; Isolated yield

4.1.2. CHARACTERIZATION OF THE COMPOUNDS:

The structures of the synthesized derivatives were constructed on the basis of their advanced spectral data. The synthetic conventional route of a novel derivatives spectral data of titled moieties was in completely agreement with designed structures. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the synthesised derivatives were identified on the basis of their chemical shifts, multiplicities, and coupling constants. The structure of the desired derivatives was constructed by the proof of spectral analysis such as ^{1}HNMR, ^{13}CNMR, LCMS and elemental analysis. In this study, proton NMR of titled compounds appeared at various values of respective groups such as hydroxyl proton, methoxy protons, , methyl protons, quaternary hydrogen as well as aromatic protons appeared at various range of values.^{13}CNMR of these derivatives are exhibited at different values. The keto group of desired compounds appeared at 169-163. ^{1}HNMR values of various protons exhibited at 8.314-7.721δppm of indole molecules, 7.214 δppm of NH_2 protons. The quaternary protons is at 4.610-4.174 δppm, the hydroxyl proton appear at 9.275 and 9.023 δppm, the methoxy protons at 3.712-3.528 δppm and methyl protons shown at 1.124-0.889 δ ppm and methylene protons at 4.088-3.913 δppm in ethycarboxylate and the protons appear at 11.974-11.067ppm.

The total observation of the entire reaction, it was reveals that the development of the desired product was obtained by subjected the catalyst. The rate of reaction of the preparative compounds were containing electron withdrawing group greater than the derivatives having electron donating group and also the compound were containing halogen group which acquired an excellent yield .The advantages of this catalyst, it was commercially available, easy handling, when it was applied into the reaction, short reaction time, and easy work up and an excellent product yields, easy to simple work-up procedure and titled products were purified by non-chromatographic process

4.2.1. ANTIMICROBIAL ACTIVITY OF COMPOUNDS (6a-6g)

The micro broth dilution method was used to assess the desired derivatives' *in vitro* antibacterial and antifungal properties. Gram-positive (*B. subtilis and S. aureus*) and gram-negative (*E. coli and P. aeruginosa)* microorganisms were used to test the invitro antibacterial activity. A. Niger and C. albicans microorganisms were used to test the invitro antifungal activity. Ketonozole and streptomycin were the standard medications used in this investigation for both antifungal and antibacterial screening. The Culture Collection and Geneank (MTCC), located in Chandigarh, India, was the commercial source of the standard strains used to screen for antibacterial and antifungal activities. Sabouraud dextrose broth was utilized for the growth of fungi and Mueller Hinton broth as a nutrient medium for bacteria. By comparing the turbidity, the inoculum size for the test strain was changed to 108 CFU/mL. Primary and secondary evaluations were used to document the outcomes. The compounds under investigation and standard medications were diluted twice to create the stock solution (2000 μg/mL).

Table-4: Antimicrobial activity screening activity synthesized scaffold (6a-6g):

Compound Code	*Zone of inhibition in (mm)					
	Bacteria				Fungi	
	S.aureus	E.coli	S. typhi	B.substill	A. Niger	C. albicans
6a	07	08	06	05	05	07
6b	20	19	17	15	12	14

6c	21	20	18	18	15	14
6d	16	19	17	15	14	15
6e	22	21	19	20	17	18
6f	23	24	21	21	19	20
6g	25	24	20	22	21	20
6h	10	12	10	12	19	18
Streptomycin	27	27	25	25	NA	NA
Fluconazole	NA	NA	NA	NA	22	22
DMSO	---	----	---	---	---	---

The process was utilized to test antioxidant activity based on ascorbic acid, the antioxidant function of some selectively generated compounds, and the DPPH stable free radical sweep impact. The demonstrates the antioxidant potential of various recently synthesized compounds against DPPH free radicals, with a high scavenging percentage and compression with ascorbic acid. The capacity of the DPPH radical to diminish was assessed using the drop in absorbance at 517 nm. Moreover, it is well known that organic molecules containing an electron-donating group (NH_2, OCH_3, and OH) that can function as free radical agents and withstand oxidation exist. The compound 1,3,4-thidiazole (1b, 1d, and 2d) has the maximum antioxidant activity, as shown in Figure 1

Table -5: Scavenging % for some of synthesized derivatives (6a-6g):

Entry	Scavenging %		
	25 mg/mL	50 mg/mL	100 mg/mL
6a	54.277	58.69	60.53
6b	69.36	79.65	84.77
6c	76.08	82.96	88.34
6d	51.22	61.54	64.22
6e	54.09	50.39	58.49
6f	46.47	51.32	54.04
6g	40.39	48.66	51.49
Ascorbic acid	85.56	90.48	95.67

The anti-oxidant evaluation of the above tale (Table 4) reveals that " 6b and 6c "compounds were displayed excellent activity and the derivatives " 6d and 6e: exhibited good activity while remaining derivatives showed low active potential.

5. CONCLUSION

In summary, the two step reaction involving dimedone, substituted aromatic aldehydes, and (E)-5-styryl-1,3,4-thiadiazol-2-amine in the presence of $Cu(OAc)_2$ under solvent as ethanol conditions has been developed to prepare a series of thiadiazolo(2,3-b)quinazolin-6-ones derivatives. This protocol is easy to follow, quick, convenient, and environmentally friendly. This method works well for synthesizing thiadiazolo (2, 3-b) quinazolin-6-ones as well and high to excellent yields, high reaction rates, avoiding toxic organic solvents, ease of operation, straightforward catalyst separation and recycling, suitability for large-scale synthetic applications. The titled analogous exhibited the two important biological properties such as antimicrobial activity as well as anti-oxidant activity

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