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CuI Promoted Efficient Synthesis and Antimicrobial Activity of Substituted 8,8-Dimethyl-5-phenyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one

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In present study, an efficient synthesis of substituted 5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one compounds promoted by CuI as catalyst was carried out. These derivatives were obtained from 1,3,4-thiadiazol-2-amine, dimedone and substituted aromatic aldehyde in presence of CuI in ethanol as solvent at 70 ºC. Initially, 1,3,4-thiadiazol-2-amine was synthesized from pyrazine-3-carboxylic acid reacted with thiosemicarbazide in the presence of 50% H_2SO_4 in acetonitrile at 70 ºC. All the newly obtained derivatives were evaluated by the spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR and LCMS and structural determination of titled analogous were analyzed by elemental analysis. The antibacterial activities of the newly synthesized compounds were also screened.

Keywords: Pyrazine-3-carboxylic acid, 1,3,4-Thiadiazol-2-amine, Dimedone, Substituted aromatic aldehyde, Antimicrobial activity.

INTRODUCTION

Fusing different heterocyclic scaffolds is a prominent field of organic synthesis for designing compounds by covalent and non-covalent linkages between various small molecules like quinazolinone, thiazole, triazole, benzofuran, imidazole, *etc*. [1]. The newly developed hybrid heterocyclics found improved pharmacological activity by interacting with biological targets [2]. The combination of heterocyclic hybrid structures of quinazolinone, thiadiazole, pyrazine, furan, pyrazole, oxazole *etc*. resulted new drug molecules which shows multi-drug resistance. The pharmacological activities of quinazolinone based hybrid drugs are reviewed widely in the literature [3,4].

Thiazolo[2,3-*b*]quinazolines are the potent functionalized molecules used in synthetic chemistry because of their capacity to be incorporated into several kinds of therapeutically useful compounds [5-9]. Quinazolinones have a wide range of therapeutic and biological effects, including antimicrobial [10-14], anti-hypertensive [15], anti-inflammatory [16], antianalegic [17], anticonvulsant [18], antioxidant [19], antitumor [20-22], cyclic-dependent kinase [23], carboys anhydrase [24]. Various catalysts were applied in different reaction such as PTA [22], transition metal free [25], palladium [26], biodegradable catalyst [27], DABCO [28], *etc*.

Thiadiazoles are found to be flexible starting materials for the synthesizing various new hybrid heterocyclic compounds because of their high prospective capacity to make them polarized push-pull systems on C-C double bond. Based on the above discussion and α,α-dioxoketen dithioacetals are used as an efficient starting material. In this regard, prolong work has been developed to synthesize novel and more effective approaches that are also environmental friendly. Our attention was on the more recent, undocumented synthesis pathways for these hybrid molecules. Initially, a pilot reaction is attempted using 5-(pyrazin-2-yl)-1,3,4-thiadiazol-2-amine (**3**), substituted aromatic aldehydes (**4**) and dimedone (**5**) in the presence of CuI catalyst. New derivatives of the synthesis of 8,8-dimethyl-5-phenyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]-thiadiazolo[2,3-*b*]quinazolin-6-one and research on the antibacterial activity supported by copper catalyst are being made

as part of our ongoing efforts. The synthesis of the aforementioned comparable compounds involved the traditional multistep chemical reactions and required numerous synthetic processes, such as the separation and purification of each step's separate products. Therefore, synthetic efficiency has been achieved by the multistep synthesis.

EXPERIMENTAL

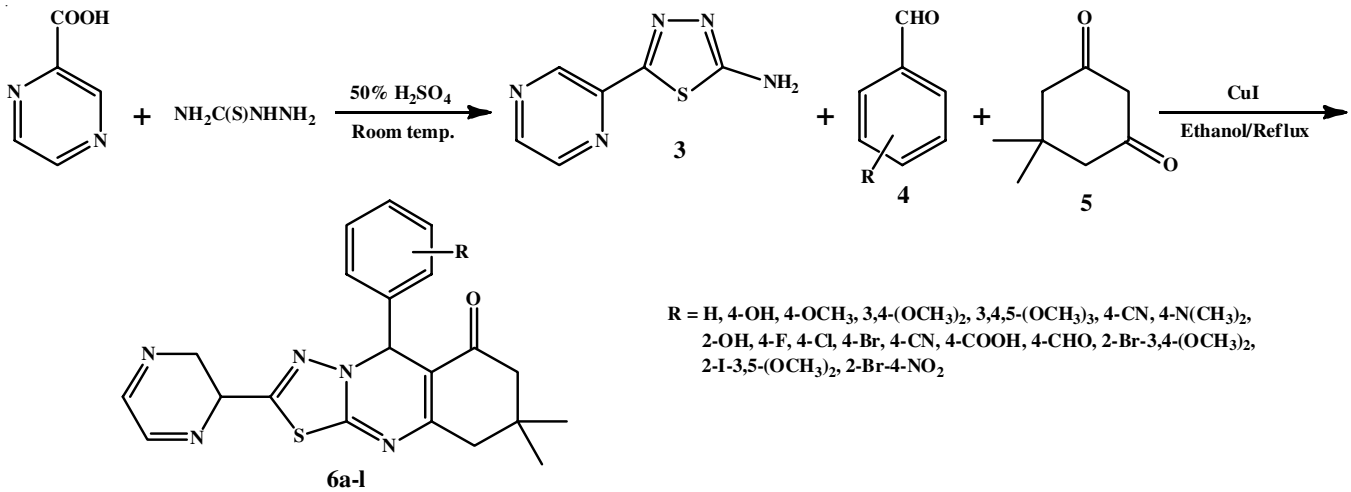
All the reagents, solvents and chemicals were purchased commercially from Sigma-Aldrich Pvt. Ltd. and the solvents without being purified were used. The melting point of the synthesized compounds was measured with the Agarwal 535 melting point instrument and are uncorrected. Thin-layer chromatography (TLC) was conducted using silica gel (Sigma-Aldrich Co.) over aluminum foil to monitor the progress of the chemical reactions. The solvents used were *n*-hexane and ethyl acetate in a 4:6 ratio and the reactions were observed under an ultraviolet (UV) light/I_2 chamber. The spectroscopic data of the novel derivatives, including ^{1}H NMR and ^{13}C NMR (400 MHz and 100 MHz), were recorded with references to TMS. The mass spectra were determined using LC–MS technique (model: Agilent 1100 series) in conjunction with the MSD. Additionally, an elemental analysis was conducted utilizing the FLASH EA 1112 CHN analyzer (Thermo-Finnigan LLC, New York, NY, USA).

Synthesis of 5-(pyrazin-2-yl)-1,3,4-thiadiazol-2-amine: Acetonitrile was introduced in 25 mL RBF and the mixture of pyrazine carboxylic acid (0.1 mol) and thiosemicarbazide (0.1 mol) was dissolved in above solvent followed by the slow addition of 50% H_2SO_4 using dropping funnel and then stirred the solution using magnetic stirrer at 70 ºC. The reaction progressed until TLC was identified as a mobile system polar and non-polar solvent (4:6). The reaction mixture cooled at 30 ºC, poured into crushed ice and neutralized with $NaHCO_3$ solution. The organic layer was separated by adding ethyl acetate and the organic layer was washed with distilled water, separated and distilled under vacuumed. Finally, desired compound was recrystallized with absolute ethanol. White solid, yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.564 (s, 1H, $C_4H_4N_2$), 8.348 (d, J = 8.8 Hz, 1H, $C_4H_4N_2$), 8.176 (d, J = 8.0 Hz, 1H, $C_4H_4N_2$), 6.663 (2H, NH_2, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 170.78, 160.09, 150.11, 143.25, 141.09, 140.28; Mass (*m/z*): 178.59 (M-H); Elemental analysis of $C_6H_5N_5S$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80), N, 39.08 (39.16).

Synthesis of substituted 8,8-dimethyl-5-phenyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo-[2,3-*b*]quinazolin-6-one: A mixture of 5-(pyrazine-2-yl)-1,3,4-thiadiazol-2-amine (0.1 mol), substituted aromatic aldehyde (0.1 mol) and dimedone (0.1 mol) dissolved in 25 mL of ethanol was taken in 50 mL of four-necked RBF. Initially the reaction initiated at room temparture for few minutes and then added copper iodide as catalyst. The reaction was heated at 70 ºC until completely consumed all the reactants and also identified the spot of reaction on the TLC plates as mobile system (ethyl acetate:*n*-hexane). The catalyst was recovered by filtration after completion of the reaction. The mixture then neutralized with solution of $NaHCO_3$ and added ethyl acetate, separated the organic layer. This organic layer washed with distilled water in twice, separated the ethyl acetate layer, distilled and vacuumed. The desired compound was recrystallized from absolute ethanol (**Scheme-I**).

8,8-Dimethyl-5-phenyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6a): Yellow solid, yield: 90%, m.p.: 210-212 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.173 (s, 1H, $C_4H_4N_2$), 8.442 (d, J = 8.0 Hz, 1H, $C_4H_4N_2$), 8.418 (d, J = 9.6 Hz, 1H, $C_4H_4N_2$), 7.495-7.276 (Ar-H, m, 5H, -C_6H_5), 4.623 (-CH-, 1H, s), 1.824 (-CH_2-, 2H, s), 1.425 (-CH_2-, s, 2H), 1.043 (3H, -CH_3, s), 0.925 (3H, -CH_3, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 191.62, 163.04, 160.29, 149.32, 143.16, 142.52, 140.32, 129.01, 128.96, 128.49, 124.44, 127.92, 60.28; Mass (*m/z*): 388.52 (M-H); ; Elemental analysis of $C_{21}H_{19}N_5OS$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80); N, 39.08 (39.16).

5-(4-Hydroxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6b): Pale-yellow solid, yield: 85%, m.p.: 225-227 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.074 (s, 1H, $C_4H_4N_2$),



Scheme-I

8.712 (s, 1H, -OH), 8.523 (d, *J* = 5.8 Hz, 1H, $C_4H_4N_2$), 8.274 (d, *J* = 11.2 Hz, 1H, $C_4H_4N_2$), 7.127 (Ar-H, d, *J* = 7.6 Hz, 2H), 6.846 (Ar-H, d, *J* = 8.8 Hz, 2H), 4.476 (-CH-, 1H, s), 1.876 (CH_2-, 2H, s), 1.665 (-CH_2-, 2H, s), 0.976 (6H, s-$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 192.38, 162.45, 160.03, 150.27, 148.61, 145.21, 143.09, 141.26, 139.58, 129.09, 128.24, 125.27, 124.04, 60.82, 48.27, 39.49, 34.33, 27.62; m.w. (*m/z*): 406.29 (M+H); Elemental analysis of $C_{21}H_{19}N_5O_2S$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80); N, 39.08 (39.16).

5-(4-Methoxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6c): Pale-yellow solid, yield: 85%, m.p.: 235-237 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.117 (s, 1H, $C_4H_4N_2$), 8.316 (d, *J* = 7.2 Hz, 1H, $C_4H_4N_2$), 8.085 (d, *J* = 10.2 Hz, 1H, $C_4H_4N_2$), 7.185 (Ar-H, d, *J* = 11.4 Hz, 1H), 7.0445 (Ar-H, d, *J* = 8.0 Hz, 1H), 4.445 (-CH-s, 1H), 3.534 (3H, OCH_3, s), 1.883 (-CH_2-, 2H, s), 1.527 (2H, -CH_2-, s), 0.987 (s, 6H, -$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 190.44, 161.95, 160.02, 155.26, 145.04, 143.72, 141.18, 139.26, 128.85, 128.16, 126.75, 125.71, 63.24, 54.09, 48.58, 38.66, 34.37, 28.24; Mass (*m/z*): 419.53 (M^+); Elemental analysis of $C_{22}H_{21}N_5O_2S$; calcd. (found) %: C, 62.99 (62.91); H, 5.05 (5.03); N, 16.69 (16.75).

5-(3,4-Dimethoxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6d): Pale-yellow solid, yield: 86%, m.p.: 241-243 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 8.997 (s, 1H, $C_4H_4N_2$), 8.440 (d, *J* = 10.6 Hz, 1H, $C_4H_4N_2$), 8.210 (d, *J* = 8.0 Hz, 1H, $C_4H_4N_2$), 7.224-7.104 (Ar-H, m, 2H), 7.021 (Ar-H, 1H, s), 4.517 (-CH-, 1H, s), 3.741 (OCH_3, 3H, s), 3.554 (s, 3H, OCH_3), 1.790 (2H, CH_2-, s), 1.410 (2H, -CH_2-, s), 0.948 (s, 6H, -$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 192. 26, 163.45, 159.11, 148.24, 145.22, 142.78, 140.17, 138.86, 132.47, 128.74, 128.14, 127.88, 126.66, 124.74, 62.57, 55.24, 48.84, 40.35, 36.22, 28.02; Mass (*m/z*): 448.36 (M-H); Elemental analysis of $C_{23}H_{23}N_5O_3S$; calcd. (found) %: C, 61.45 (61.40); H, 5.16 (5.15); N, 15.58 (15.64).

8,8-Dimethyl-2-(pyrazin-2-yl)-5-(3,4,5-trimethoxyphenyl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]-quinazolin-6-one (6e): White solid, yield: 87%, m.p.: 254-256 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.072 (s, 1H, $C_4H_4N_2$), 8.364 (d, *J* = 7.6 Hz, 1H, $C_4H_4N_2$), 8.065 (d, *J* = 11.2 Hz, 1H, $C_4H_4N_2$), 6.886 (Ar-H, d, *J* = 8.8 Hz, 2H), 4.497 (-CH-, 1H, s), 3.673 (OCH_3, 3H, s), 3.586 (6H, s, $(OCH_3)_2$), 1.775 (CH_2-, 2H, s), 1.508 (-CH_2-, 2H, s), 1.023 (-CH_3, 6H, s), 0.947 (-CH_3, 3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 189.84, 161.78, 158.62, 153.81, 147.06, 144.53, 143.09, 134.07, 129.14, 128.92, 128.48, 127.88, 124.44, 63.62, 58.08, 55.84, 49.02, 38.47, 32.15, 28.25. Mass (*m/z*): 480.04 (M-H); Elemental analysis of $C_{24}H_{25}N_5O_4S$; calcd. (found) %: C, 60.11 (60.06); H, 5.25 (5.30); N, 14.60 (14.66).

4-(8,8-Dimethyl-6-oxo-2-(pyrazin-2-yl)-6,7,8,9-tetrahydro-5*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-5-yl)-benzonitrile (6f): White solid, yield: 92%, m.p.: 194-196 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.225 (s, 1H, $C_4H_4N_2$), 8.328 (d, *J* = 11.2 Hz, 1H, $C_4H_4N_2$), 8.102 (d, *J* = 7.4 Hz, 1H, $C_4H_4N_2$), 7.665 (Ar-H, d, *J* = 7.2 Hz, 1H), 7.454 (Ar-H, d, *J* = 8.8 Hz, 1H), 4.623 (-CH-, s, 1H), 1.884 (-CH_2-, 2H, s), 1.507 (s, 2H, -CH_2-), 1.077 (-CH_3, 3H, s), 0.956 (-CH_3, 3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 196.91, 160.78, 158.09, 145, 17, 143.07, 140.26, 137.68, 133.19, 130.08, 128.92, 128.04, 123.11, 117.64, 65.49, 48.27, 38.02, 30.37, 27.64; Mass (*m/z*): 415.41 (M+H); Elemental analysis of $C_{22}H_{18}N_6OS$; calcd. (found) %: C, 63.75 (63.70); H, 4.38 (4.37); N, 20.28 (20.34).

5-(4-(Dimethylamino)-2-hydroxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo-[2,3-*b*]quinazolin-6-one (6g): Yellow solid, yield: 86%, m.p.: 197-199 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.464 (s, 1H, -OH), 9.115 (s, 1H, $C_4H_4N_2$), 8.562 (d, *J* = 8.8 Hz, 1H, $C_4H_4N_2$), 8.274 (d, *J* = 7.6 Hz, 1H, $C_4H_4N_2$), 7.166 (Ar-H, 1H, s), 6.874 (Ar-H, d, *J* = 8.0 Hz, 2H), 4.374 (-CH_2-, s, 1H), 2.489 (s, 6H, $(CH_3)_2$), 1.864 (-CH_2-s, 2H), 1.502 (-CH_2-, 2H, s), 1.177 (CH_3, 3H, s), 0.965 (CH_3, s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 193.79, 162.46, 159.02, 152.33, 145.82, 143.53, 141.65, 139.75, 168.09, 134.33, 130.09, 129.64, 129.12, 128.46, 126.39, 57.26, 48.83, 42.39, 38.46, 30.71, 28.02. Mass (*m/z*): 433.04 (M+H); Elemental analysis of $C_{23}H_{24}N_6OS$; calcd. (found) %: C, 61.59 (61.54); H, 5.39 (5.38); N, 18.74 (18.79).

5-(4-Fluorophenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6h): Pale-yellow solid, yield: 90%, m.p.: 187-189 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.196 (s, 1H, $C_4H_4N_2$), 8.663 (d, *J* = 8. Hz, 1H, $C_4H_4N_2$), 8.394 (d, *J* = 10.6 Hz, 1H, $C_4H_4N_2$), 7.396 (Ar-H, d, *J* = 12.2 Hz, 2H), 7.255 (Ar-H, d, *J* = 8.0 Hz, 2H), 4.492 (-CH-, 1H, s), 1.972 (-CH_2-, 2H, s), 1.665 (-CH_2-, 2H, s), 0.988 (s, 6H, -$(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 194.66, 162.75, 160.04, 155.76, 147.06, 145.11, 143.38, 140.22, 134.21, 130.36, 129.04, 128.33, 126.02, 64.75, 49.82, 39.45, 30.24, 26.96. Mass (*m/z*): 408.54 (M+H); Elemental analysis of $C_{21}H_{18}N_5OSF$; calcd. (found) %: C, 61.90 (61.85); H, 4.45 (4.44); N, 17.19 (17.24).

5-(4-Chlorophenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6i): Pale brown solid, yield: 91%, m.p.: 192-194 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.224 ($C_4H_4N_2$, 1H, s), 8.587 ($C_4H_4N_2$, *J* = 7.2. Hz, 1H), 8.314 ($C_4H_4N_2$, *J* = 5.6 Hz, 1H, d), 7.426 (Ar-H, *J* = 6.8 Hz, 2H, d), 7.296 (Ar-H, *J* = 7.8 Hz, 2H, d), 4.52 (-CH-, 1H, s), 1.896 (-CH_2-, 2H, s), 1.627 (-CH_2-, 2H, s), 1.074 (-CH_3, 3H, s), 0.955 (CH_3, 3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 191.76, 161.09, 158.66, 154.37, 146.07, 144.74, 142.32, 140.14, 136.17, 133.74, 130.62, 128.92, 128.04, 63.47, 49.66, 38.67, 30.62, 26.96. Mass (*m/z*): 425.31 (M+2); ; Elemental analysis of $C_{21}H_{18}N_5OSCl$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80); N, 39.08 (39.16).

5-(4-Bromophenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6j): Pale red solid, yield: 91%, m.p.: 204-206 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.118 ($C_4H_4N_2$, 1H, s), 8.510 ($C_4H_4N_2$, *J* = 8.0 Hz, 1H, d), 8.147 ($C_4H_4N_2$, *J* = 9.6 Hz, 1H, d), 7.594 (Ar-H, *J* = 5.8 Hz, 1H, d), 7.074 (Ar-H, d, *J* = 8.8 Hz, 2H), 4.610 (-CH-, 1H, s), 1.894 (2H, -CH_2-, s), 1.494 (s, 2H, -CH_2-), 1.144 (6H, s, $(CH_3)_2$), ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 194.74, 160.86, 157.84, 151.09, 145.56, 144.02, 141.66, 138.37, 135.06, 129.47, 128.04, 124.62, 65.71, 47.48, 41.02, 29.02, 27.06; Mass (*m/z*): 469.22 (M+2); Elemental analysis of $C_{21}H_{18}N_5OSBr$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80); N, 39.08 (39.16).

4-(8,8-Dimethyl-6-oxo-2-(pyrazin-2-yl)-6,7,8,9-tetrahydro-5*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-5-yl)benzoic acid (6k): White solid, yield: 93%, m.p.: 187-189 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.276 ($C_4H_4N_2$, 1H, s), 8.576 ($C_4H_4N_2$, *J* = 10.6 Hz, d, 1H), 8.223 ($C_4H_4N_2$, *J* = 8.0 Hz, 1H, d), 7.724 (Ar-H, *J* = 5.4 Hz, 1H, d), 7.583 (Ar-H, d, *J* = 8.8 Hz, 1H, d), 4.674 (-CH-, s, 1H), 1.924 (-CH_2-, 2H, s), 1.606 (-CH_2-, s, 2H), 0.943 (6H, s, $(CH_3)_2$); ^{13}C NMR (100 MHz, CDCl3) δ ppm: 195.84, 165.58, 161.09, 159.78, 145.08, 144.11, 143.61, 140.27, 133.72, 129.05, 128.68, 128.45, 127.66, 123.86, 63.44, 49.02, 38.74, 30.81, 27.04, Mass (*m/z*): 433.37 (M+); Elemental analysis of $C_{22}H_{19}N_5O_3S$; calcd. (found) %: C, 40.22 (40.15); H, 2.81 (2.80); N, 39.08 (39.16).

5-(2-Bromo-3,4-dimethoxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo-[2,3-*b*]quinazolin-6-one (6l): Pale red, yield: 85%, m.p.: 215-217 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.078 ($C_4H_4N_2$, 1H, s), 8.667 ($C_4H_4N_2$, *J* = 7.6 Hz, d, 1H), 8.215 ($C_4H_4N_2$, *J* = 9.6 Hz, d, 1H), 7.114 (Ar-H, *J* = 8.0 Hz, d, 1H), 6.946 (Ar-H, *J* = 10.6 Hz, d, 1H), 4.412 (1H, -CH-, s), 3.714 (-OCH_3, 3H, s), 3.556 (-OCH_3, 3H, s), 1.896 (2H, -CH_2-, s), 1.574 (2H, -CH_2-, s), 0.975 (s, 6H, $(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 193.09, 163.15, 160.04, 153.62, 150.05, 145.66, 143.42, 141.64, 138.96, 135.02, 132.33, 129.72, 124.26, 122.22, 120.32, 62.92, 58.63, 55.74, 48.39, 38.44, 30.22, 26.68: Mass (*m/z*): 529.37 (M+2); Elemental analysis of $C_{23}H_{22}N_5O_3SBr$; calcd. (found) %: C, 59.50 (59.44); H, 4.28 (4.27); N, 16.52 (16.58).

5-(4-Chlorophenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6i): Pale brown solid, yield: 91%, m.p.: 192-194 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.224 (s, 1H, $C_4H_4N_2$), 8.587 (d, *J* = 7.2. Hz, 1H, $C_4H_4N_2$), 8.314 (d, *J* = 5.6 Hz, 1H, $C_4H_4N_2$), 7.426 (Ar-H, d, *J* = 6.8 Hz, 2H), 7.296 (Ar-H, d, *J* = 7.8 Hz, 2H), 4.52 (-CH-, 1H, s), 1.896 (-CH_2-, 2H, s), 1.627 (-CH_2-, 2H, s), 1.074 (-CH_3, 3H, s), 0.955 (CH_3, 3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 191.76, 161.09, 158.66, 154.37, 146.07, 144.74, 142.32, 140.14, 136.17, 133.74, 130.62, 128.92, 128.04, 63.47, 49.66, 38.67, 30.62, 26.96. Mass (*m/z*): 425.31 (M+2); Elemental analysis of $C_{21}H_{18}N_5OSCl$; calcd. (found) %: C, 59.50 (59.44); H, 4.28 (4.27); N, 16.52 (16.58).

5-(4-Bromophenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-6-one (6j): Pale red solid, yield: 91%, m.p.: 204-206 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.118 (s, 1H, $C_4H_4N_2$), 8.510 (d, *J* = 8.0 Hz, 1H, $C_4H_4N_2$), 8.147 (d, *J* = 9.6 Hz, 1H, $C_4H_4N_2$), 7.594 (Ar-H, d, *J* = 5.8 Hz, 1H), 7.074 (Ar-H, d, *J* = 8.8 Hz, 2H), 4.610 (-CH-, 1H, s), 1.894 (2H, -CH_2-, s), 1.494 (s, 2H, -CH_2-), 1.144 (6H, s, $(CH_3)_2$), ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 194.74, 160.86, 157.84, 151.09, 145.56, 144.02, 141.66, 138.37, 135.06, 129.47, 128.04, 124.62, 65.71, 47.48, 41.02, 29.02, 27.06; Mass (*m/z*): 469.22 (M+2); Elemental analysis of $C_{21}H_{18}N_5OSBr$; calcd. (found) %: C, 53.85 (53.80); H, 3.87 (3.85); N, 14.95 (15.02).

4-(8,8-Dimethyl-6-oxo-2-(pyrazin-2-yl)-6,7,8,9-tetrahydro-5*H*-[1,3,4]thiadiazolo[2,3-*b*]quinazolin-5-yl)benzoic acid (6k): White solid, yield: 93%, m.p.: 187-189 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.276 (s, 1H, $C_4H_4N_2$), 8.576 (d, *J* = 10.6 Hz, 1H, $C_4H_4N_2$), 8.223 (d, *J* = 8.0 Hz, 1H, $C_4H_4N_2$), 7.724 (Ar-H, d, *J* = 5.4 Hz, 1H), 7.583 (Ar-H, d, *J* = 8.8 Hz, 1H), 4.674 (-CH-, s, 1H), 1.924 (-CH_2-, 2H, s), 1.606 (-CH_2-, s, 2H), 0.943 (6H, s, $(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 195.84, 165.58, 161.09, 159.78, 145.08, 144.11, 143.61, 140.27, 133.72, 129.05, 128.68, 128.45, 127.66, 123.86, 63.44, 49.02, 38.74, 30.81, 27.04; Mass (*m/z*): 433.37 (M+); Elemental analysis of $C_{22}H_{19}N_5O_3S$; calcd. (found) %: C, 60.96 (60.90); H, 4.42 (4.40); N, 16.16 (16.21).

5-(2-Bromo-3,4-dimethoxyphenyl)-8,8-dimethyl-2-(pyrazin-2-yl)-5,7,8,9-tetrahydro-6*H*-[1,3,4]thiadiazolo-[2,3-*b*]quinazolin-6-one (6l): Pale red, yield: 85%, m.p.: 215-217 ºC, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.078 (s, 1H, $C_4H_4N_2$), 8.667 (d, *J* = 7.6 Hz, 1H, $C_4H_4N_2$), 8.215 (d, *J* = 9.6 Hz, 1H, $C_4H_4N_2$), 7.114 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.946 (d, *J* = 10.6 Hz, 1H, Ar-H), 4.412 (s, 1H, -CH-), 3.714 (s, 3H, -OCH_3), 3.556 (s, 3H, -OCH_3), 1.896 (s, 2H, -CH_2-), 1.574 (s, 2H, -CH_2-), 0.975 (s, 6H, $(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 193.09, 163.15, 160.04, 153.62, 150.05, 145.66, 143.42, 141.64, 138.96, 135.02, 132.33, 129.72, 124.26, 122.22, 120.32, 62.92, 58.63, 55.74, 48.39, 38.44, 30.22, 26.68, Mass (*m/z*): 529.37 (M+2); Elemental analysis of $C_{23}H_{22}N_5O_3SBr$; calcd. (found) %: C, 52.28 (52.23); H, 4.20 (4.19); N, 13.25 (13.32).

Antimicrobial activity: The newly synthesized derivatives (**6a-l**) were tested for their *in vitro* antibacterial activity using the cup-plate method. For antibacterial growth, we used a variety of pathogenic strains, including *Pseudomonas aeruginosa*, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus*, *B. substills* (Gram-positive). The nutrient agar (NMA) was used to assess the antimicrobial activity of the tested derivatives by keeping the neutral pH of medium. The NMA was sterilized for 45 min at 37 °C and 15 lbs of pressure in an autoclave. A 20 mL of sterilized NAM was placed into petri dishes in a laminar air flow to solidify. Following the solidification, 100 µL of examined bacteria pathogens were injected into NMA. The investigated compounds were dispersed in DMSO and Whatmann filter paper No. 1 disks were used to cover NAM plates that had been bacterially infected and then left to incubate at 37 ºC in 24 h. The similar zones of inhibition investigated were contrasted with ciprafloxin also.

For antifungal assay, *Aspergillus flavus*, *Candida albicans* and *Aspergillus niger* were used. The micromycetes were grown on malt agar and stored at 4 ºC. The antimicrobial activity of the synthesized compounds was evaluated using the micro-dilution method for determining the minimal inhibitory and minimal fungicidal concentrations [29]. The fungal spores were removed from the agar plates by rinsing them with sterile 0.85% saline solution containing 0.1% Tween 80 (v/v). The spore suspension was diluted with sterile saline to a concentration of around 1.0×10^5 in a final volume of 100 µL each well. The inocula were kept at 4 ºC for future use. The inocula dilutions were grown on solid malt agar to confirm the absence of contamination and validate the inoculum. The minimum inhibitory concentration (MIC) was determined using a serial dilution approach in 96-well microtiter plates. The compounds studied were dissolved in DMSO at a concentration of 1 µg/mL and then introduced to a broth malt medium containing inoculum.

The microplates were incubated at 28 ºC for 72 h. The minimum inhibitory concentrations (MICs) were determined as the lowest doses where no observable growth was observed.

RESULTS AND DISCUSSION

Novel derivatives of the target compounds *viz*. thiadiazolo-[2,3-*b*]quinazolin-6-one were synthesized by proposing a novel synthetic route. Compounds **6a-l** can be obtained from the mixture of 5-(pyrazine-2-yl)-1,3,4-thiadizole-2-amine, substituted aromatic aldehydes and 5,5-dimethyl cyclohexan-1,3-dione (dimedone) in the presence of CuI as catalyst in ethanol at 70 ºC. Initially, when the reaction was carried out at room temperature without catalyst, no reaction progress was observed. The reaction proceeded once the catalyst was added, and the temperature was gradually increased to 70 ºC.

The catalyst is important in facilitating the synthesis process until the reaction attained completion. Various copper halides, *e.g*. $CuCl_2$, $CuBr_2$ and CuI, were utilized as catalysts to enhance the maximum yield of desirable compounds. The CuI catalyst functioned to achieve a high yield (Table-1). It was also observed that an dosage of catalyst also effect the progress of the reaction. However, after the addition of 0.5 mol catalyst, ~20% yield of product was observed. The yield further improved after increasing the catalyst amount to 1.0 and 1.5 mol. A 94% yield was achieved by adding 1.5 mol of catalyst to obtain the product. Moreover, there was no enhancement observed when an excessive amount of catalyst was introduced as indicated in Table-2.

The solvents also play an important factor to obtain the maximum yield of desired product. During the reaction and the percentage of the synthesized derivatives manly depend on the solubility of the reactants. Among the polar aprotic and protic solvents examined in this procedure *e.g*. DMF, acetonitrile, ethanol and methanol, only ethanol proved to be the most effective and dependable (Table-3).

TABLE-1
THE OPTIMIZATION OF VARIOUS COPPER CATALYSTS FOR THE SYNTHESIS OF DERIVATIVES (**6i**)

Entry	Catalyst	Yield (%)	Time (min)
1	$CuCl_2$	72%	240
2	$CuBr_2$	79%	150
3	CuI	94%	120

TABLE-2
SCREENING OF THE CATALYTIC USING LOADED CATALYST ACCOUNTABLE FOR THE SYNTHESIS OF DERIVATIVES (**6i**)

Entry	Loaded catalyst	Yield (%)	Time (min)
1	0.5	20	180
2	1.0	40	150
3	1.5	94	75
4	2.0	94	90
5	2.5	94	120

TABLE-3
SCREENING OF THE CATALYTIC USES VARIOUS SOLVENTS ACCOUNTABLE FOR THE SYNTHESIS OF DERIVATIVES (**6i**)

Entry	Solvent	Yield (%)	Time (min)
1	DMF	50	210
2	Acetonitrile	45	180
3	Ethanol	94	120
4	Methanol	75	150

Antibacterial activity: The *in vitro* bactericidal activity of the synthesized compounds **6a-l** was compared with the standard streptomycin. Most of the synthesized analogues exhibited potent action against all the tested bacterial strains, as indicated in Table-4. Compounds **6d** and **6i** showed strong antibacterial action against Gram-positive bacteria *viz. E. coli*, *P. aeruginosa*, and Gram-negative bacteria *e.g*. *B. subtilis* and *S. aureus*. Compounds **6c**, **6h** and **6l** exhibited strong antibacterial activity, whereas compounds **6b**, **6f** and **6g** exhibit considerable moderate activity. Only compounds **6a**, **6e** and **6j** had low activity against bacterial strains due to the presence high electron withdrawing group. These findings indicated that compounds with electron withdrawing groups and electron donating groups showed moderate to good activity when compared. Moreover, the halogen-containing compound exhibited exceptional antibacterial activity.

TABLE-4
THE *in vitro* ANTIMICROBIAL ACTIVITIES OF TITLED DERIVATIVES

Compound	Antibacterial activity				Antifungal activity		
	Gram-positive bacteria		Gram-negative bacteria		*Aspergillus favus*	*Aspergillus niger*	*Candida albicans*
	Escherichia coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*			
6a	10	09	13	12	06	05	08
6b	13	15	18	16	11	13	11
6c	20	19	21	21	12	11	12
6d	23	24	26	25	19	21	20
6e	04	06	08	06	19	18	19
6f	12	14	16	15	11	13	10
6g	12	13	15	17	07	10	12
6h	20	21	22	22	10	12	07
6i	24	23	25	26	20	21	21
6j	08	05	09	07	17	18	18
6k	14	15	17	17	10	13	09
6l	21	20	22	22	08	11	10
Streptomycin	27	27	30	30	–	–	–
Ketonozole	–	–	–	–	22	22	22

Synthesized compounds **6a-l** showed different values for *in vitro* antifungal activity against *A. favus*, *A. niger* and *C. albicans*. It was observed that aromatic aldehydes with a specific functional group were dependent on the parent derivatives. Compounds **6d**, **6e** and **6i** exhibited exceptional potency due to their electron-releasing moiety, whereas compound **6j** demonstrated good activity against fungal strains. Compounds **6c**, **6f** and **6k** displayed a moderate activity due to their less electron-releasing characteristics (Table-4). Compounds **6a**, **6g** and **6l** exhibited weakly activity due to their electron withdrawing character.

Conclusion

In summary, a efficient method for the synthesis of thiadiazolo[2,3-*b*]quinazolin-6-ones *via* a three-component one-pot reaction of 5-(pyrazin-2-yl)-1,3,4-thiadiazol-2-amine (**3**), substituted aromatic aldehydes (**4**) and dimedone (**5**) in the presence of copper iodide catalyst in ethanol as solvent at 70 ºC was achieved with excellent yield. The synthesized compounds were evaluated for their *in vitro* antibacterial activity against antibacterial and antifungal strains. The synthesized compounds demonstrated excellent antibacterial activity against Gram-positive organisms in comparison to compounds tested against Gram-negative organisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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