Tandem Synthesis And Antibacterial Studies Of Novel 3-Substituted Tetrahydrobenzo [4,5] Thieno [1,2,4] Triazolo [4,3-B] Pyridazine And 2- (5-(Substituted) -4h-1,2,4-Triazole-3-Yl) -Tetrahydrobenzo [B] Thiophene-3-Carbonitirile Derivatives

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ABSTRACT: A series of novel 3-substituted tetrahydrobenzo [4,5] thieno [1,2,4] triazolo [4,3-*b*] pyridazine and 2- (5- (substituted) -4H-1,2,4-triazole-3-yl) -4,5,6,7-tetrahydrobenzo [b] thiophene-3-carbonitrile derivative were synthesized. The structures of all derivatives were characterized by LCMS, ^{1}H NMR ^{13}C NMR spectroscopy. Newly synthesized compounds (**8a-8f)** and **(9**a-**9h**) were subjected for their invitro antibacterial screening against Bacillus substilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli R. solanacearum Among the tested compounds, compounds **7d, 7f** and **8f** exhibited significant antibacterial activity.

KEYWORDS: 1,2,4-triazolopyridazine, 6,7,8,9-tetrahydrobenzothienopyridazine, Boronic acid, and Antibacterial activity.

1. INTRODUCTION

Azines and azoles incorporated drugs were found to be more admired in the field of medicinal chemistry due to their potential biological and pharmacological activities. Among the heterocyclic compounds, pyridazine is one such compound and their innumerable applications have been attributed significantly because of its diverse biological activities including antimicrobial, antifungal, antiviral, antitumor, antihypertensive, antitubercular and anticancer activities [1-12]. Besides, in the last few decades, the chemistry of 1, 2, 4- triazoles and their fused heterocyclic have received considerable attention owing to their numerous biological activities such as antifungal, antibacterial, analgesics, anti-inflammatory, antineoplastic, antiviral, sedatives, anxiolytics, anti-convulsants, antimigraine, antihistaminics and other activities. Morever, the wide variety of biological application of 1,2,4-triazole motifs have also been extremely explored for antitubercular, serotonergic, anti-allergy, CNS depressant and anti-inflammatory respectively [13-20].

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Interestingly, the fusion of homocyclic or heterocyclic ring with 1,2,4-triazole triazole moiety exhibits noteworthy activities for instance, the triazolothienopyrimidinone and pyrazolotriazolopyrimidine compounds were found as xanthine oxidase inhibitor and adenosine A_{2A}/A_3receptors antagonists[21-22] and the 5,6,7,8-tetrahydro [1] benzothieno [2,3-d] pyrimidin-4 (3H) -one derivatives possess anticonvulsant activities[23]. Likewise, triazolopyridazine derivatives are extensively used as a ligand for $GABA_A$ and adenosine receptors[24-28]. Inspired by the biological activities of triazoles and pyridazines, our attempts were made to fuse two or more heterocyclic ring with pyridazine system in order to investicate the usefulness of this system. The purpose of this study was to synthesize and evaluate the biological importance of novel 6,7,8,9-tetrahydro [1] benzothieno [2,3-d] pyridazine with 1,2,4-triazole moiety.

2. EXPERIMENTAL PROTOCOLS

2.1. Materials and methods

All reagents were purchased from commercial suppliers and were used without purification. Melting points were determined in Buchi B-545 melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance and Varian -400 & 300 NMR MHz spectrometers in DMSO-d_6 & $CDCl_3$ solution using TMS as an internal reference and ^{13}C NMR spectra were recorded on 100 & 75 MHz. Mass spectra were recorded on GC-MS. Carbon, Hydrogen, and Nitrogen were analyzed on Elementor instrument. All these compounds were purified by flash column Chromatography using 230-400 mesh silicagel.

2.1.1. 3-Formyl-4,5,6,7-tetrahydro-1-benzothiophene-2-carboxylic acid (2)

4,5,6,7-Tetrahydro [b] enzothiophene-2-carboxylic acid (27.4 mmol) was dissolved in 75 ml of dry THF and cooled to -65 to -70^{o}C under nitrogen atmosphere, then 24 ml of n-BuLi (60 mmol) was slowly added over a period of 1h at -65^{o} C. The resulting mixture was stirred at the same temperature for further 2h. Dimethyl formamide (13.7 mmol) was slowly added to the reaction over 0.5h. Stirring was continued at -60^{o}C for 2h. The reaction mixture was slowly warmed to -15^{o}C and stirring was continued for further 4h. The reaction mass was quenched with 50 ml of HCl (1.5 N) and extracted with 100 ml of DCM. The organic layer was evaporated, the crude was recrystallised from methanol to afford compound **2** Yield 87% ; yellow solid; m.p.: 182-188^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 1.94-1.93 (m, 4H, -CH_2CH_2) , 2.81-2.79 (m, 2H, -CH_2) , 2.93-2.91 (m, 2H, -CH_2) , 8.10 (s, 1H, Ar-H) , 10.30 (s, 1H, -CHO) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ =158.5, 146.89, 139.0, 133.6, 130.9, 25.6, 23.4, 22.9, 21.6 ppm; MS: m/z = 209 [M^{+}-1]

2.1.2. 3-Formyl-4,5,6,7-tetrahydro-1-benzothiophene-2-carboxylate (3)

Oxalyl chloride (57.0 mmol) was added dropwise to a ice cold solution of compound **2** (47.5mmol) in 200 ml of DCM. The resulting mixture was stirred at RT for 1h, and then ethanol (237.8 mmol) was slowly added to the reaction mixture and heated at 50 ^{o}C for 2 h. The reaction mass was quenched with 75 ml of sodium bicarbonate solution (10 %,) , extracted with 300 ml of DCM. The organic layer was evaporated, the crude was purified by column chromatography to afford titled compound **3** as a pale yellow liqui. Yield 70% ; ^{1}H NMR (300 MHz, $DMSOd_6$) : δ =1.31-1.22 (t, 3H, J = 7.0 Hz, -CH_3) , 1.73-1.67 (m, 4H, -CH_2CH_2) , 2.77-2.70 (m, 4H, -CH_2CH_2) , 4.34-4.27 (q, 2H, J = 7.0 Hz, -OCH_2) , 10.4 (s, 1H, -CHO) ppm; ^{13}C NMR (75MHz, $CDCl_3$) : δ = 188.6, 161.2, 145.3, 142.8, 141.6, 137.1, 136.1ppm MS: m/z = 237 [M^{+}-1]

2.1.3. 6,7,8,9-Tetrahydro [1] benzothieno [2,3-d] pyridazin-4 (3H) -one (4)

Method: (**a**) A mixture of compound **2** (95.1 mmol**)** and hydrazine hydrate (98%, 475.0 mmol) was dissolved in 200 ml of ethanol and refluxed for 24 h. After removal of the solvent in vacuo, the precipitated solid was filtered, washed with water, dried to afford compound **4** as a off white solid. yield 76%; mp 276- 278.5 ^{o}C. Method (**b**) A mixture of compound **3** (84.1 mmol**)** and hydrazine hydrate (98%, 419.6 mmol) was dissolved in 200 ml of ethanol and refluxed for 24 h. After removal of the solvent in vacuo, the precipitated solid was filtered, washed with water, dried to afford compound **4** as a off white solid. Yield 81%; m.p.: 277- 278.5 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) : δ = 1.76-1.69 (m, 4H,-CH_2CH_2) , 2.77-2.72 (m, 4H, -CH_2CH_2) , 10.5 (s, 1H, CH=N) , 12.84 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) : δ = 189.0, 162.8, 142.6, 141.3, 138.2, 137.2ppm. MS: m/z **=** 207 [M+1] .

2.1.4 .4-Chloro-6,7,8,9-tetrahydro [1] benzothieno [2,3-d] pyridazine (5)

A mixture of compound **4** (121.5 mmol) and $POCl_3$ (728.1 mmol) in 250 ml of acetonitrile was heated at 90^{o}C for 6 h. After evaporation the solvent and $POCl_3$ by high vacuuo, the residue was diluted with 150 ml of DCM , poured into ice-water and basified with saturated sodium carbonate solution and the organic layer was evaporated and purified by column chromatography to afford compound **5** as a pale yellow solid. Yield 74% ; m.p.: 199.7-205.4 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) ; δ = 2.04-2.03 (m, 4H, -CH_2CH_2) , 3.1-2.98 (m, 4H, -CH_2CH_2) , 10.09 (s, 1H, CH=N) ppm ; ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 21.0, 22.4, 23.6, 26.2, 134.1, 138.8, 139.2, 143.9, 150.4, 152.9 ppm; MS: m/z = 225 [M+1] .

2.1.5. (3-Bromophenyl) -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] triazolo [4,3] pyridazine (7)

Amixture of **5** (44.5 mmol) , m- bromobenzoic hydrazide (53.4 mmol) and few drops of $POCl_3$ were dissolved in 100 ml of n- butanol and the mixture was refluxed at 130 ^{o}C for 12 h. The reaction mass was diluted with 500 ml of DCM, then poured in to ice-water, basified with saturated sodium carbonate solution and the organic layer was evaporated and purified by purified by recrystallisation in methanol to afford **7** Yield: 64%; yellow solid; m.p.: 263.2-266.1 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.98 (m, 4H, -CH_2CH_2) , 2.89-2.92 (m, 2H, -CH_2) , 2.95-2.94 (m, 2H, -CH_2) , 7.44-7.38 (t, 1H, J = 7.86 Hz, Ar-H) , 7.63-7.61 (d, 1H, J = 7.83 Hz, Ar-H) , 8.47-8.44 (d, 1H, J = 7.83 Hz, Ar-H) , 8.57 (s, 1H, Ar-H) , 8.71 (s, 1H, CH=N) ; ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 21.6, 22.9, 23.4, 25.3, 122.5, 126.1, 128.4, 130.0, 130.1, 130.3, 130.8, 132.8, 139.5, 142.3, 146.7, 162.8 ppm; MS**:** m/z = 386 [M+1] .

2.3 General procedure for the synthesis of 3- [substituted biphenyl-3-yl] -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] triazolo [4,3-b] pyridazine (7a-7g)

A mixture of compound **7** (2.6 mmol) , Potassium phosphate tribasic (7.8 mmol) , boronic acid (3.1mmol) , tricyclohexylphosphine (0.64 mmol) in anhydrous DMF (20 mL) was purged with argon for 20 mins, then, palladium acetate trimer (0.11 mmol) was added. The resulting mixture was reaction heated at 100 ^{o}C for 6 h. The solvent was removed under reduced pressure; the residue was quenched with water and extracted with 30 % solution of methanol in chloroform. The combined organic layer was washed with water, dried over sodium sulphate and evaporated. The crude was purified by recrystallisation from the mixture of 1, 2-dimethoxy ethane and hexane.

2.4 General procedure for the synthesis of 2.4 2- (5-(Substituted– [1,1'-biphenyl] -3-yl) -4H-1,2,4-triazole-3-yl) -4,5,6,7-tetrahydrobenzo [b] thiophene-3-carbonitirile (8a-8g)

A mixture of compound **7** (2.6 mmol) , Potassium phosphate tribasic (7.8 mmol) , boronic acid (3.1mmol) , tricyclohexylphosphine (0.64 mmol) in anhydrous DMF (20 mL) was purged with argon for 20 mins, then, palladium acetate trimer (0.11 mmol) was added. The resulting mixture was reaction heated at 100 ^{o}C for 12 h. The solvent was removed under reduced pressure; the residue was quenched with water and extracted with 30 % solution of

methanol in chloroform. The combined organic layer was washed with water, dried over sodium sulphate and evaporated. The crude was purified by recrystallisation from the mixture of 1, 2-dimethoxy ethane and hexane.

ANALYTICAL DATAS

2.3.1. 1- [3'- (7,8,9,10-Tetrahydro [1] benzothieno [2,3-d] [1,2-4] triazolo [4,3-b] pyridazine-3-yl) biphenyl-4-yl] ethanone (7a)

Yield 66%; brown solid; m.p.: 269-273 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 2.02-2.00 (m, 4H, -CH_2CH_2) , 2.67 (s, 3H, -$COCH_3$) , 2.96-2.94 (m, 2H, CH_2) , 3.00-2.99 (t, 2H, -CH_2) , 7.70-7.66 (m, 1H, Ar-H) , 7.83-7.79 (m, 3H, Ar-H) , 8.10-8.08 (d, 2H, J = 2.00 Hz Ar-H) , 8.68-8.54 (m, 1H, Ar-H) , 8.79 (s, 1H, CH=N) , 8.80 (d, 1H, J=1.56, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) : δ = 21.6, 22.9, 23.4, 25.3, 26.6, 126.6, 127.3, 127.4, 128.6, 128.8, 129.1, 130.1, 130.3, 130.8, 136.0, 139.5, 140.1, 142.1, 143.5,145.0, 147.9 197.6 ppm; MS: m/z 425 = (M+1)

3- (4-Ethyl- [1,1'-biphenyl] -3y) -7,8,9,10-tetrahydro [1] ben*zo [4,5]* thieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7*b*)

Yield 70%; brown solid; m.p.: 245-250 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) : δ = 1.3 (t, 3H, J=15.1Hz, -CH_3) , 2.0 (s, 4H, --CH_2CH_2) , 2.73 (q, 2H, J=7.1Hz -CH_2) , 2.96-3.01 (m, 4H, -CH_2) , 7.34 (d, 2H, J=7.8 Hz, Ar-H) , 7.65 (d, 2H, J=7.2 Hz, Ar-H) , 8.46 (d, 1H, J=7.6 Hz, Ar-H) , 8.69 (s, 1H, Ar-H) , 8.74 (s, 1H, CH=N) , 8.80 (d, 1H, J=1.56 Hz, Ar-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) : δ = 15.4, 21.6, 22.9, 23.4, 25.3, 28.4, 126.3, 126.4, 126.9, 127.1, 128.2, 128.4, 128.9, 13.0, 130.3, 130.7, 137.9, 141.4, 141.9, 14.0, 143.6, 148.2.ppm; MS: m/z 411 = (M+1) .

2.3.2. 7,8,9,10-Tetrahydro-3- [3- (pyrazin-2-yl) phenyl] thieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7*c*)

Yield 64% ; brown solid; m.p.: 269-273 ºC ; ^{1}H NMR (300 MHz $CDCl_3$) : δ = 2.02-1.98 (m, 4H, -CH_2CH_2) , 2.91-2.90 (m, 2H, -CH_2) , 2.99-2.95 (m, 2H, -CH_2) , 7.72-7.71 (m, 2H, Ar-H) , 8.64-8.61 (m, 2H, Ar-H) , 8.80-8.79 (d, 1H, J= 0.93 Hz, Ar-H) , 9.07 (s, 1H, Ar-H) , 9.25 (s, 1H, CH=N) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 21.6, 22.9, 23.4, 25.3, 126.1, 27.8, 128.1, 128.2, 129.7, 130.1, 130.3, 130.8, 133.8, 134.6, 139.6, 142.3, 143.2, 147.4, 154.9, 157.7 ppm; MS: m/z = 383 (M-1)

2.3.4. 3- [2'Methoxymethylbiphenyl-3-yl] -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7*d*)

$C_{25}H_{22}N_4OS$) Yield 67% ; white solid; m.p.: 149-152 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.22 (m, 4H, -CH_2CH_2) , 2.68-2.64 (m, 4H, -CH_2CH_2) , 3.41 (m, 3H, OCH_3) , 4.44 (s, 2H, -OCH_2) , 7.51-7.31 (m, 6H, Ar-H) , 7.67-7.64 (d, 1H, J=7.86 Hz, Ar-H) , 8.02-7.99 (d, 1H, J=7.74 Hz, Ar-H) , 8.25 (s, 1H, CH=N) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 21.7, 22.7, 24.2, 24.7, 58.1, 107.5, 115.2, 125.2, 125.6, 126.3, 126.9, 128.8, 129.0, 129.2, 137.6, 138.7, 140.1, 141.4, 148.3, 142.5,158.5, , ppm; MS: m/z = 427 (M^++1)

2.3.5. 3- [2'Ethoxybiphenyl-3-yl] -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7*e*)

Yield 66% ; Off white solid; m.p.: 219-222 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.39-1.30 (m, 3H, -CH_3) , 1.98-1.97 (m, 4H, -CH_2CH_2) , 2.88 -2.92 (m, 2H, -CH_2) , 2.94-2.98 (m, 2H, -CH_2) 4.12-4.05 (m, 2H, OCH_2) , 7.08-7.00 (m, 2H, Ar-H) , 7.36-7.26 (m, 1H, Ar-H) , 7.47-7.44 (d, 1H, J = 1.62, Ar-H) , 7.61-7.56 (t, 1H, J = 7.80, Ar-H) , 7.75-7.72. (d, 1H, J=7.83, Ar-H) , 8.45-8.42 (d, 1H, J=7.86, Ar-H) , 8.54 (s, 1H, Ar-H) , 8.71 (s, 1H, CH=N) ppm; ^{13}C NMR (75 MHz, $DMSOd_6$) : δ = 14.7, 21.6, 22.9, 23.4, 25.3, 64.0, 112.6, 120.8, 126.2, 128.0, 128.7, 128.9, 129.9, 130.2, 130.7, 130.9, 131.3, 138.9, 139.2, 141.9, 142.9, 148.4, 155.8 ppm; MS: m/z = 427 (M^++1)

2.3.6.3- [4'- (Benzyloxy) -biphenyl-3-yl] -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7*f*)

Yield 71% ; white solid; m.p.: 228-230 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.98-2.11 (s, 4H, -CH_2CH_2) , 2.89-2.93 (m, 2H, CH_2) , 2.96 -2.94 (m, 2H, -CH_2) , 5.12 (s, 2H, -OCH_2) , 6.91-6.80 (m, 2H, Ar-H) , 7.51-7.33 (m, 7H, Ar-H) , 7.69-7.58 (m, 2H, Ar-H) , 8.47-8.44 (m, 2H, J = 7.65 Hz, Ar-H) , 8.58 (s, 1H, Ar-H) , 8.65 (s, 1H, CH=N) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 21.7, 22.9, 23.4, 25.3, 70.3, 102.8, 103.2, 111.1, 121.0, 120.9,126.5, 126.7, 127.4, 128.1, 130.09, 130.2, 130.4, 130.7, 131.0, 136.1, 136.3, 139.4, 142.1, 144.2, 148, 159.2 ppm; MS: m/z = 489 (M+1)

2.3.7.3- (2',5'Dichlorobiphenyl-3-yl) -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (7g)

Yield 68%; white solid; m.p.: 251-252 ºC ^{1}H NMR (400 MHz $CDCl_3$) : δ = 1.99-1.98 (m, 4H, -CH_2CH_2) , 2.79-2.97 (m, 4H, -CH_2CH_2) , 7.31-7.26 (m, 1H, Ar-H) , 7.46-7.40 (m, 2H, Ar-H) , 7.66-7.53 (m, 2H, Ar-H) , 8.60-8.55 (m, 1H, Ar-H) 8.6 (s, 1H, CH=N) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) :δ = = 21.7, 23.0, 23.5, 23.4, 126.79, 127.5, 128.5, 128.5, 128.7, 130.1, 130.4, 130.8, 130.9, 131.0, 131.2, 132.6, 138.5, 139.5, 141.4, 142.1, 143.1, 147.9, 156.5 ppm; MS: m/z = 452 (M^++1)

2.3.8.2-5- (4'-Methyl- [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl-) 4,5,6,7-tetrahydrobenzo [b] thiophene-3-carbonitrile (8a)

Yield 70% ; white solid; m.p.: 230-235 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.87-1.85 (m, 4H, -CH_2CH_2) , 2.73-2.70 (m, 4H, -CH_2CH_2) , 3.79 (s, 3H, -CH_3) , 7.05-6.97 (s, 2H, Ar-H) , 7.36-7.31 (m, 2H, Ar-H) , 7.51-7.46 (m, 1H, Ar-H) , 7.64-7.62 (d, 1H, Ar-H) , 7.98-7.96 (d, 1H, J = 7.65 Hz, Ar-H) , 8.16 (s, 1H, CH=N) , 12.3 (s, 1H, -NH) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 21.8, 22.8, 22.3, 24.7, 55.5, 107.5, 111.2, 115,2, 120.8, 125.2, 127.7, 128.5, 128.9, 129.5, 130.7, 131.7, 137.6, 138.2, 139.2, 156.3 ppm; MS: m/z = 384 (M+1)

2.3.9. 2-5- (4'-Isopropyl - [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl-) 4,5,6,7-tetrahydrobenzo [b] -thiophene-3-carbonitrile] - (8b)

Yield 72% ; white solid; m.p.: 238-242 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.27 (d, 6H, J = 6.9 Hz, $-CH_2CH_2$) , 1.80 (m, 4H, $-CH_2CH_2$) , 2.62 (m, 4H, $-CH_2$ CH_2) , 2.89-2.96 (m, 1H, Ar-H) , 7.26-7.21 (m, 1H, Ar-H) , 7.38-7.33 (m, 1H, Ar-H) , 7.43-7.52 (m, 1H, Ar-H) , 7.67-7.65 (d, 1H, Ar-H) , 8.03-8.01 (d, 1H, Ar-H) , 8.2 (s, 1H, CH=N) , 12.8 (s, 1H, -NH) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 14.0, 21.7, 22.5, 22.7, 23.9, 24.2, 24.6, 31.5, 34.1, 58.9, 107.4, 115.2, 124.5, 125.1, 125.3, 125.5, 125.5, 128.7, 129.0, 129.1, 137.5, 139.9, 141.9, 149.4 ppm; MS: m/z = 425 (M+1) .

2.3.10. Methyl 3– (5- (3-cyano-4,5,6,7-tetrahydrobenzo [b] thieophen-2-yl) -4H-1,2,4-triazol-3yl) - [1,1' biphenyl] -2-carboxylate (8c)

Yield 73% ; white solid; m.p.: 240-245 ºC ; ^{1}H NMR (400 MHz, $DMSOD_6$) : δ = 1.78 (m, 4H, $-CH_2CH_2$) , 2.62 (m, 2H,
$-CH_2$) , 2.73 (m, 2H, $-CH_2$) , 3.8 (m, 3H, OCH_3) , 7.70-7.64 (m, 2H, Ar-H) , 7.87-7.84 (m, 1H, Ar-H) , 8.05-7.98 (m, 3H, Ar-H) , 8.33-7.28 (m, 2H, Ar-H) , 7.67-7.65 (d, 1H, Ar-H) , 8.03-8.01 (d, 1H, Ar-H) , 14.9 (s, 1H, -NH) ppm; ^{13}C NMR (100 MH,z $DMSOD_6$) : δ = 21.8, 22.8, 24.4, 24.6, 52.7, 107.3, 114.9, 124.8, 126.3, 127.6, 127.8, 129.0, 129.3, 130.1, 130.5, 130.9, 132.0, 137.7, 138.0, 140.2, 140.3, 166 ppm; MS: m/z = 441 (M+1)

2.3.11.2- (5'- (4- (Benzyloxy) - [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl)] -4,5,6,7-tetrahydro [b] benzothiophene-3-carbonotrile (8d)

Yield 70% ; white solid; m.p.: 228-230 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.79 (m, 4H, $-CH_2CH_2$) , 2.64 (m, 2H, CH_2) , 2.74 (m, 2H, $-CH_2$) , 5.17 (s, 2H, $-OCH_2$) , 6.95-6.89 (m, 2H, Ar-H) , 7.18-7.14 (m, 1H, Ar-H) , 7.28-7.23 (m, 3H, Ar-H) , 7.58-7.39 (m, 3H, Ar-H) , 7.66-7.60 (m, 2H, Ar-H) , 7.97-7.94 (d, 1H, Ar-H) , 8.22 (s, 1H, Ar-H) , 14.83 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 21.8, 22.8, 24.4, 24.6, 70.5, 101.6, 108.0, 114.9, 125.2, 126.0, 126.7, 127.8, 128.2, 128.7, 129.4, 131.9, 132.0, 136.9, 137.7, 138.0, 138.5, 156.2, 156.7, 156.8, 161.5, 164.7 ppm; MS: m/z = 489 (M+1)

2.3.12.2- (5'- (2- (Metghoxy) - [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl)] -4,5,6,7-tetrahydro [b] benzothiophene-3-carbonotrile (8e)

Yield 75% ; white solid; m.p.: 220-222 ºC ; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 1.88-190 (m, 4H, $-CH_2CH_2$) , 2.78-2.74 (m, 4H, $-CH_2CH_2$) , 3.82 (s, 3H, $-OCH_3$) , 7.07-6.99 (m, 2H, Ar-H) , 7.38-7.33 (m, 2H, Ar-H) , 7.55-7.49 (m, 1H, Ar-H) , 7.67-7.64 (m, 1H, Ar-H) , 8.00-7.97 (d, 1H, *J*=9Hz Ar-H) , 8.15 (s, 1H, Ar-H) , 8.22 (s, 1H, Ar-H) , 12.52 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz $CDCl_3$) : δ = 21.8, 22.8, 24.3, 24.7, 55.5, 107.5, 111.2, 120.8, 125.2, 127.6, 128.5, 128.9, 129.5, 130.7, 131.7, 138.2, 139.2, 156.3 ppm; MS: m/z = 413 (M+1)

2.3.13. 2- (5'- (4- (Benzyloxy) -3'-fluoro- [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl)] -4,5,6,7-tetrahydro [b] benzothiophene-3-carbonotrile (8f)

Yield 73% ; white solid; m.p.: 230-235 ºC ; ^{1}H NMR (400 MHz, $DMSOD_6$) : δ = 1.82-180 (m, 4H, $-CH_2CH_2$) , 2.65-63 (m, 2H, $-CH_2$) , 2.76-2.75 (m, 2H, $-CH_2$) , 5.18 (s, 2H, $-OCH_2$) , 6.95-6.91 (m, 1H, Ar-H) , 7.18-7.15 (m, 1H, Ar-H) , 7.28-7.24 (m, 3H, Ar-H) , 7.59-7.41 (m, 3H, Ar-H) , 7.95-7.61 (m, 2H, Ar-H) , 7.96 (s, 1H, Ar-H) , 8.23 (s, 1H, Ar-H) , 14.82 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz $DMSOD_6$) : δ = 21.4, 22.4, 24.0, 24.3, 70..0,101.5, 107.4, 107.6, 114.5, 124.8, 126.3, 127.8, 128.3, 129.0, 131.5, 136.5, 137.3, 137.6, 155.2, 155.8, 156.3, 156.4, 160.1, 161.5, 163. ppm; MS: m/z = 507 (M+1)

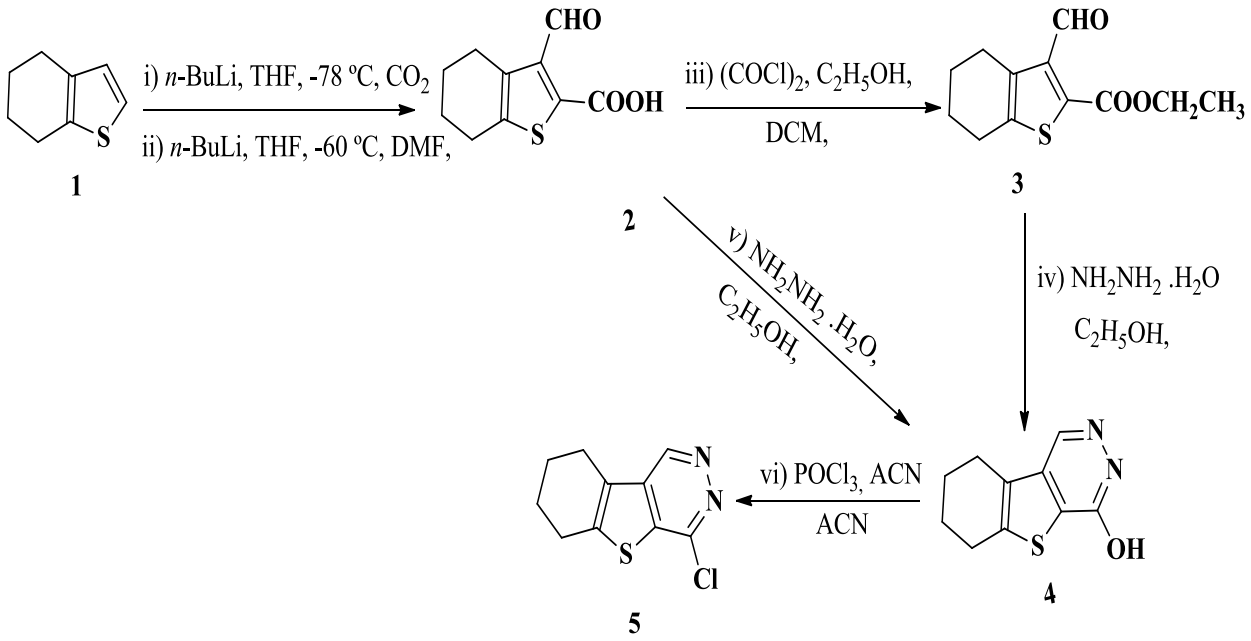
2.3.14.2- (5– (3,difluoro- [1,1'biphenyl] -3-yl) -4H-1,2,4-triazol-3yl)] -4,5,6,7-tetrahydro [b] benzothiophene-3-carbonotrile (8g)

Yield 75% ; white solid; m.p.: 228-230 ºC ; ^{1}H NMR (300 MHz, *$DMSO_6$*) : δ = 1.82-1.80 (m, 4H, $-CH_2CH_2$) , 2.64 (m, 2H, CH_2) , 2.75 (m, 2H, $-CH_2$) , 7.33-7.28 (m, 1H, Ar-H) , 7.54-7.51 (m, 2H, Ar-H) , 7.70-7.66 (m, 1H, Ar-H) , 7.90-7.92 (d, 1H, Ar-H) , 78.06-8.04 (d, 2H, Ar-H) , 8.34 (s, 1H, Ar-H) , 14.89 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz *$DMSO_6$*) : δ = 21.4, 22.3, 23.9, 24.2, 78.7, 103, 106.7, 109.8, 110.1, 114.4, 124.6, 126.5, 129.0, 130.0, 137.2, 137.5, 138.3, 138.6, 142.9, 154.9, 154.8, 164.0, 164.2 ppm; MS: m/z = 489 (M+1) .

3. RESULTS AND DISCUSSION

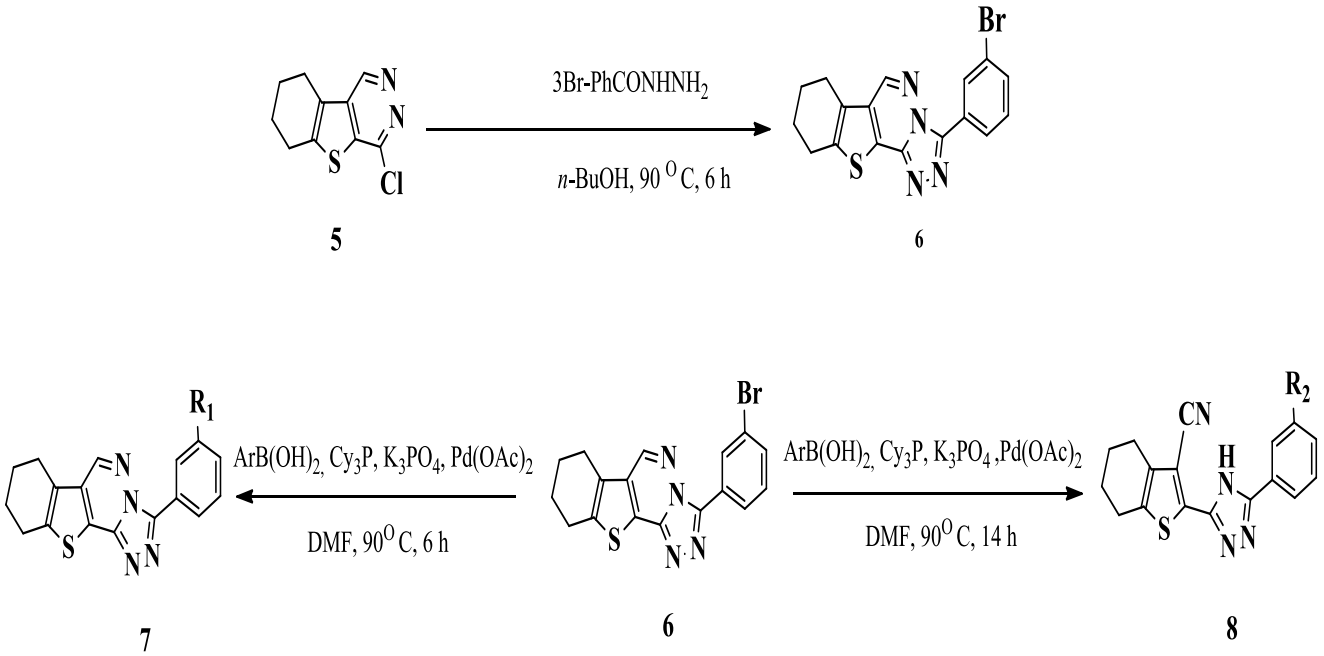
3.1. Chemistry

The 3-Formyl-4,5,6,7-tetrahydro-1-benzothiophene-2-carboxylic acid (**2**) was synthesized from 4,5,6,7-tetrahydro-1-benzothiophene (**1**) according to the literature procedure[29]. Compound **2** was prepared from the treatment of corresponding acid chloride and ethanol. 4-Hydroxy-6,7,8,9-tetrahydro [1] benzothieno [2,3-d] pyridazine (**4**) was directly synthesized by the treatment of 3-formyl-4,5,6,7-tetrahydro [1] benzothiophene-2-carboxylic acid (**2**) with hydrazine hydrate in ethanol under reflux.



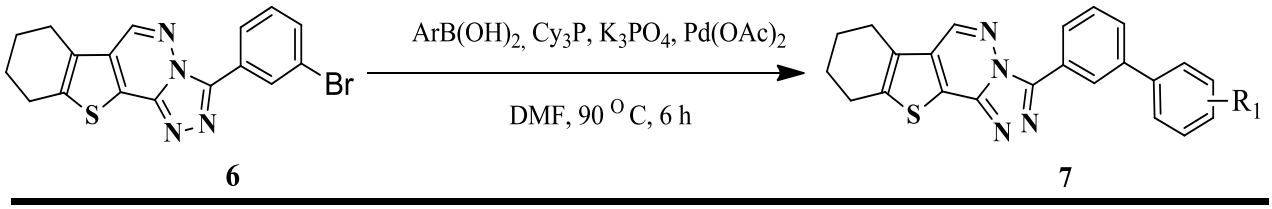
Scheme 1 *Synthesis of 4-Chloro-6,7,8,9-tetrahydrobenzo [4,5] thieno [2,3] pyriazine*

The compound (**4**) can be alternatively prepared by the treatment 3-formyl-4,5,6,7-tetrahydro [1] benzothiophene-2-carboxylic acid (**2**) with hydrazine hydrate in ethanol. Compound **4** on treatment with $POCl_3$ in acetonitrile at 90°C yielded 4-chloro-6,7,8,9-tetrahydro [1] benzothieno [2,3-d] pyridazine (**5**) in good yield.

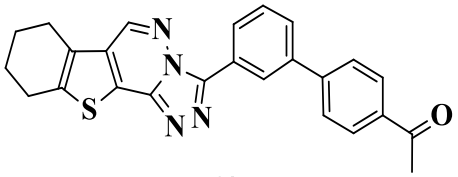


Scheme 2 *Synthesis of 8a-8f and 8a-8k series of derivatives*

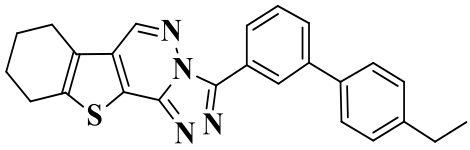
Table 1 *Synthesis of compound* ***7a-7g*** *series.*



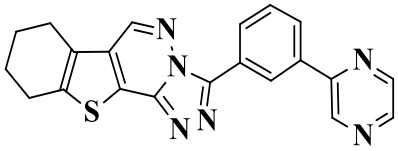
6 **7**



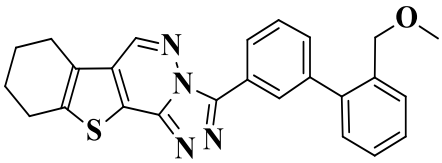
7a, 66%



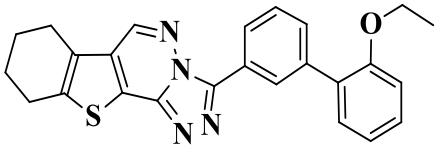
7b, 70%



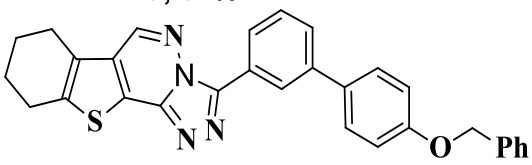
7c, 64%



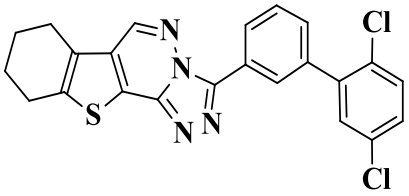
7d, 67%



7e, 66%



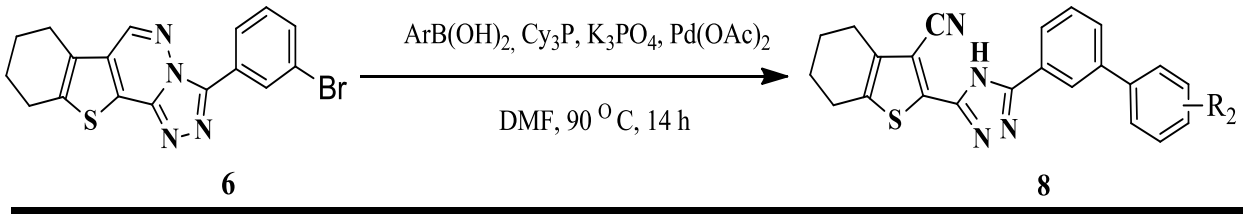
7f, 71%

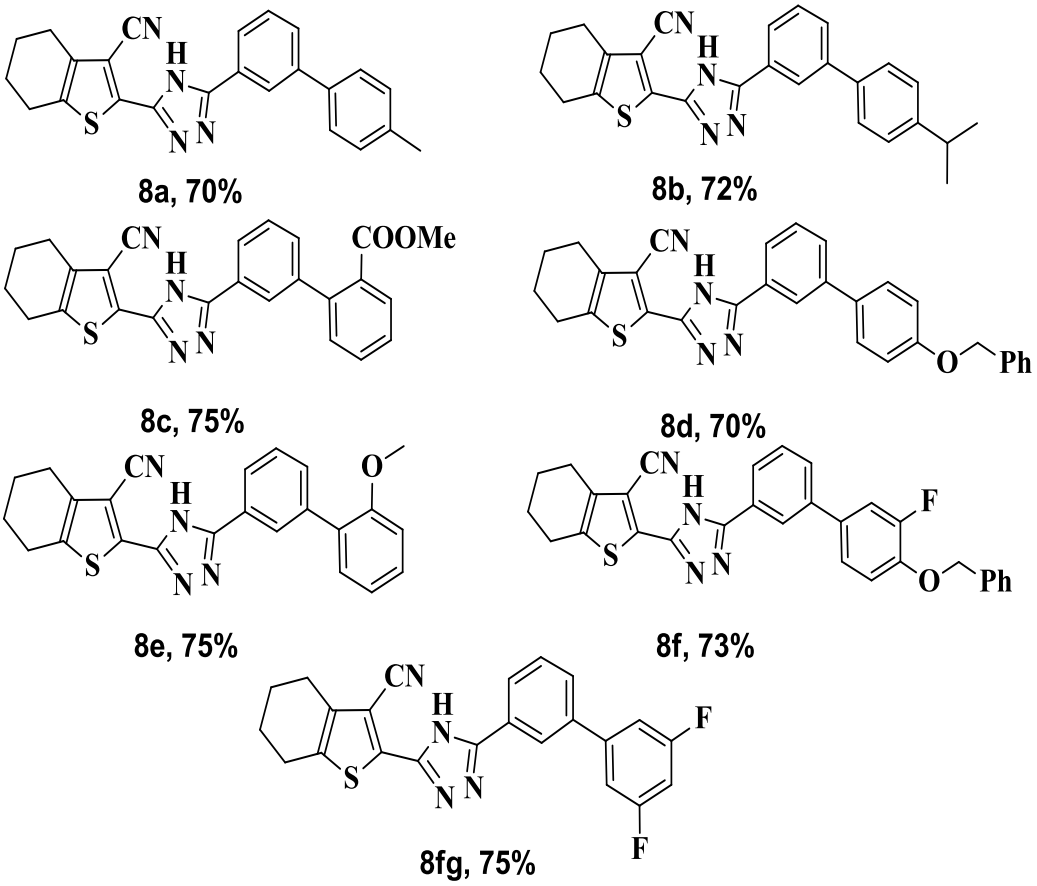


7fg, 73%

Reaction Condition : Compound 7, Potassium phosphate tribasic (7.8 mmol) , boronic acid (3.1mmol) , tricyclohexylphosphine (0.64 mmol) in anhydrous DMF (20 mL) was purged with argon for 20 mins, then, palladium acetate trimer (0.11 mmol) was added and heated at 90^0 C for 6 h. Initially, our plan was to derive the derivatives of 7 series, in order to achieve these derivatives (7a-7g) , the 4-Chloro-6,7,8,9-tetrahydro [1] benzothieno [2,3-d] pyridazine (5) was heated with 3-bromobenzoic hydrazide in n-BuOH to afford 3- (3-bromophenyl) -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] triazolo [4,3-b] pyridazine (6) which on further

Table 1 *Synthesis of compound* ***8a-8g*** *series.*





Reaction Condition : Compound 7, Potassium phosphate tribasic (7.8 mmol) , boronic acid (3.1mmol) , tricyclohexylphosphine (0.64 mmol) in anhydrous DMF (20 mL) was purged with argon for 20 mins, then, palladium acetate trimer (0.11 mmol) was added and heated at 90^0 C for 14 h. treatment with various aryl boronic acid furnished the series of 3- [substitutedbiphenyl-3-yl] -7,8,9,10-tetrahydro [1] benzothieno [2,3-d] triazolo [4,3-b] pyridazine derivatives (Table 3) . Surprisingly the reaction was allowed to heat for prolong time approximately more than 12 hr afforded the 8a-8g compounds Table 2. Both series compound were clearly identified NMR and LCMS spectroscopy and the reaction sequences are outlined in Scheme 1 and 2.

3.2. Antimicrobial Activity

All the synthesized compounds were subjected to in-vitro antibacterial screening test against both Gram-positive (S. Aureus and B. Subtilis) and Gram-negative bacteria (E.Coli and K. Pneumonia) using disc diffusion method[30-32]. Screening results revealed that the compounds **7a-g**and **8a-g** showed good to moderate activities towards the tested microorganism. The antibacterial screening results are given in the **Table 1.**

Table 3 *Compounds exhibit moderate In vitro-Antibacterial activities against the bacterial strains.*

Compounds	**Inhibition zone diameter (mm/mg sample)**				
	S. aureus B. subtiis E. coli K. pneumonia R. solanacearum				
7a	6	6	**9**	b	9
7b	7	6	**10**	**9.5**	**10**
7c	**7**	7	**9**	8	9
7d	**11**	**12**	**12**	**11**	**11**
7e	7	8	6	8	**10**
7f	**10.6**	**10**	**11**	**10**	8
7g	**9**	**10**	**10**	**10**	8
8a	9	8	9.2	9	7
8b	8.7	8.5	**9**	**9**	**9**
8c	**9**	**10**	8	7	11
8d	**10**	**9**	5	8	**10**
8e	**10**	**10**	**10**	**11**	**8**
8f	**12.0**	**12**	**11**	**11**	**7**
8g	**9**	**9**	7.8	7	**9**
Amphicilin	**12**	**14**	**14**	**12**	**12**

The data generated from antibacterial screening revealed that the compounds of **7a-7g**showed weak activities while compounds of **8a-8g** displayed strong activity towards the tested microorganism. The results indicated in the **Table 1** shows that compounds **7d, 7f, 7g, 8e** and **8f** have exhibited significant activities against both Gram positive and Gram negative bacteria and the plant bacteria of R. solanacearum. Among all the tested compounds **7d**, **7f** and **8f** showed good to strong activity against the tested strains. In addition, the compound **6a, 6b, and 6e** have moderate activities only on Klebsiellae Pneumoniae and Escherica coli whereas the compound**s 8c, 8d** and **8g** have significant activities towards Bcillus substills and Staphylococus aureus (**Table 3**).

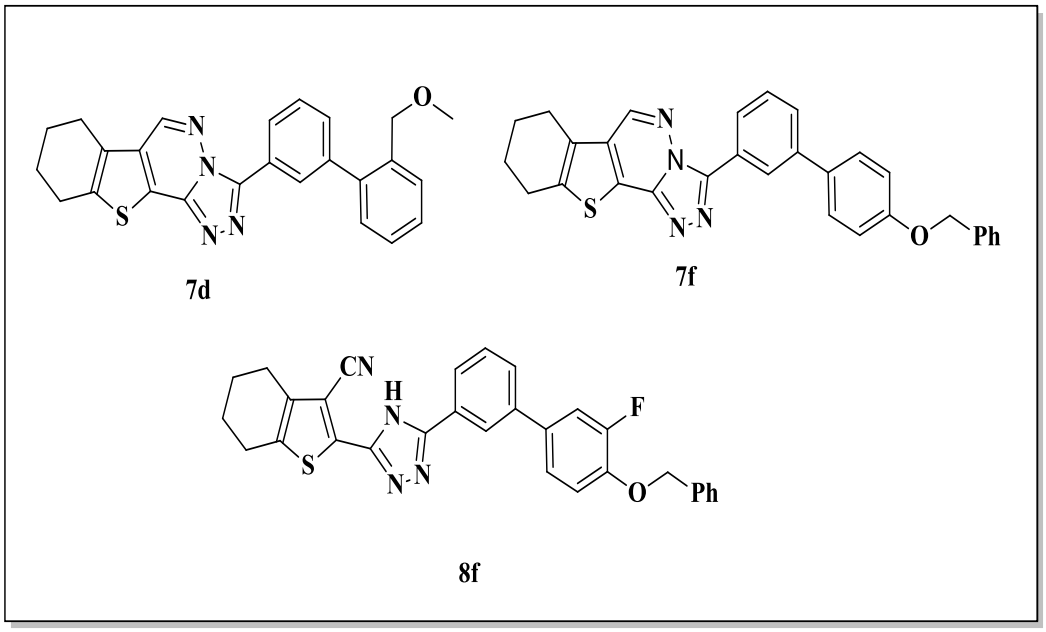


FIGURE 1. *MORE ACTIVE COMPOUND AMONG THE NEWLY SYNTHESIZED DERIVATIVES*

All the compounds were also subjected to their antifungal activities against the fungi namely, Aspergillus flavus and Aspergillus Niger. Fluconazole was used as standard for the comparison of antifungal activity of the compounds **7** and **8** series**.** This result indicated that almost all the compounds have week antifungal activity against the tested fungi. Furthermore the more active derivatives are shown in the **Figure1.**

4. CONCLUSIONS

In conclusion, we have synthesized a novel series of Novel [2,3-d] [1,2,4] triazolo [4,3-b] pyridazine (**7a-g**) and 2- (5-(Substituted – [1,1'-biphenyl] -3-yl) -4H-1,2,4-triazole-3-yl) -4,5,6,7-tetrahydrobenzo [b] thiophene-3-carbonitirile (**8a-g**) conveniently in good yields. The structures of all compounds were confirmed on the basis of spectral data and elemental analysis. All newly synthesized compounds were tested for their antibacterial activity. Among the tested compounds, compounds **7d**, **7f** and **8f** displayed good inhibition against the tested bacterial strains.

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