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LETTERS

Synthesis, antimicrobial and antimalarial study of novel 1,3,4-thiadiazole derivatives incorporating imidazo [1,2-b] pyridazine and thiazolidinone moieties

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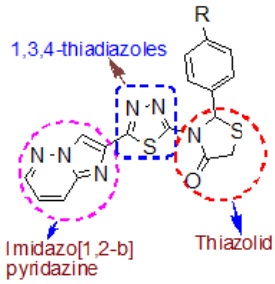
ABSTRACT



Structure	Antibacterial Activity (MIC) µg/ml	Antimalarial Activity (IC50)µg/ml
8a	62.5 (E.Coli)	0.67
8b	100 (P.aeruginosa)	0.56
Ampicillin	100 (E.Coli, P.aeruginosa)	0.268 (Quinine standard)
Chloramphenicol	50 (E.Coli, P.aeruginosa)	

R = Cl (8a)
R = F (8b)

A new class of 1,3,4-thiadiazole derivatives **8a-i** incorporating imidazo [1,2-b] pyridazine and thiazolidinone moieties were synthesized by the reaction of arylidene derivatives of 1,3,4-thiadiazoles 2-amine having imidazo [1,2-b] pyridazine moiety (Schiff base) **7a-i** with thioglycolic acid. Schiff base **7a-i** were synthesized by the reaction of 1,3,4-thiadiazoles 2-amine having imidazo [1,2-b] pyridazine moiety **6** with substituted benzaldehyde. The compounds were screened for in-vitro antimicrobial activity against two gram positive (*Streptococcus Pyogenes* and *Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) as well as for antifungal and antimalarial activity against *plasmodium falciparum* strain. Compound **8a** and **8b** exhibited good antimicrobial and antimalarial activity.

Keywords: Imidazo [1,2-b] pyridazine, 1,3,4-Thiadiazoles, Thiazolidinone, Antimicrobial activity, Antimalarial activity

INTRODUCTION

The resistance towards available drugs is rapidly becoming a major problem worldwide. There is need to design new compounds to deal with this increasing resistance and it has become one of the most important areas of research today. Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as "hydrogen binding domain" and "two-electron donor system". It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide,[1] methazolamide,[2] megazol,[3] etc. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporins, hence can be used in antibiotic preparations.

Currently, imidazopyridazines are in the focus of attention of researchers due to their diverse biological activities. Anticancer,[4] anticonvulsant (antiepileptic),[5] antimalarial[6] effects as well as stimulating activity on soluble guanylate cyclase (relief of angina pectoris),[7] activity against human immunodeficiency virus[8] and influenza[9] have been identified for these compounds. Ponatinib, a third-generation tyrosine kinase inhibitor has been implemented in clinical practice for the treatment of chronic myeloid leukemia. Among the possible alternatives of systems where imidazole and pyridazine cycles are fused of greatest interest in terms of biological activity are imidazo[1,2-b] pyridazine[10,11] and imidazo[4,5-d] pyridazine[12,13] heterocyclic systems.

Similarly 1,3-thiazolidin-4-ones are heterocyclic nucleus that have an atom of sulfur and nitrogen at position 1 and 3, respectively and a carbonyl group at position 4 have been

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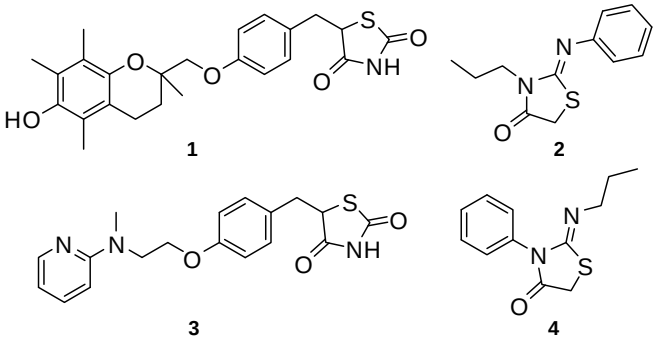
subjected to extensive study in the recent years. The 4-thiazolidinone scaffold is very versatile and has featured in a number of clinically used drugs. They have found uses as antitubercular, antimicrobial, anti-inflammatory and as antiviral agents, especially as anti-HIV agents.[8,19] Many biologically active products having thiazolidinones are used in medicine for the treatment of various diseases, e.g. Troglitazone **1** and Rosiglitazone **2** used as insulin sensitizing drugs for the treatment of type-2 diabetes.[13] 2-Imino-4-thiazolidinones, **3** and **4**, proved to have interesting anti-inflammatory activity.[14-16] It has been extensively reported that presence of arylazo,[17] sulfamoylphenylazo[18] or phenylhydrazono,[19] moieties at different positions of the thiazolidone ring enhanced anti-microbial activity and its antibacterial activity may be due to its inhibitory activity of enzyme Mur B which is a precursor acting during the biosynthesis of peptidoglycan.[20] Numerous reports have appeared in the literature which highlight their chemistry and pharmacological uses.[21-23]



Looking at the importance of these heterocyclic nuclei, it is thought of interest to accommodate 1,3,4-thiadiazole, imidazo[1,2-b] pyridazine with thiazolidinones moieties in single molecular framework and screen them for their various biological activities.

RESULT AND DISCUSSION

CHEMISTRY

In the present work, 2-(aryl)-3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one **8a-i**, were synthesized by using 3-amino-6-chloro pyridazine as a starting material. 3-amino-6-chloro pyridazine **1** on cyclisation reaction with ethyl 3-bromo pyruvate resulted ethyl 6-chloroimidazo[1,2-b]pyridazine-2-carboxylate **2**, which on hydrogenation reaction with Pd/C gave ethyl imidazo[1,2-b]pyridazine-2-carboxylate **3**. Compound **3** on heating reaction with hydrazine hydrate resulted imidazo[1,2-b]pyridazine-2-carbohydrazide **4**, which on refluxing with trimethyl silyl isothiocyanate gave 2-(imidazo[1,2-b]pyridazine-2-carbonyl)hydrazinecarbothioamide **5**. Compound **5** on cyclisation reaction with conc. sulfuric acid resulted 5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-amine **6**. When compound **6** was refluxed with various substituted benzaldehydes it gave the corresponding arylidene derivatives (Schiff base) **7a-i**, which on treatment with thioglycolic acid gave desired thiazolidinone derivatives **8a-i**.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of all the synthesized compounds were tested in-vitro against pathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus Pyogenes* and the results were compared with standard drugs (Ampicillin and Chloramphenicol). In case of *S.aureus* compounds **8a**, **8b**, **8e**, **8f**, **8g** and **8h** exhibit good activity while compound 8i shows moderate activity and rest of the compounds possess less activity. In case of *S. pyogenus* compound **8f** exhibit good activity while **8d** and **8g** shows moderate activity and rest of the compound possess less activity. In case of *E. coli* Compounds**8a** and **8b** shows higher activity and compounds **8c**, **8d** and **8h** exhibit good activity while rest of the compounds possess less activity. In case of *P.aeruginosa* compounds **8a, 8b** and **8d** exhibit good activity than the rest of the compounds. The results are given in Table-1.

ANTIFUNGAL ACTIVITY

The antifungal activity of all the synthesized compounds were tested in-vitro against fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* and the results were compared with standard drugs (Nystatin and Greseofulvin). In case of *C. Albicans* compounds **8a**, **8b** and **8d** exhibit good activity while **8c**, **8e**, **8f**, **8h** and **8i** show moderate activity and rest of the compounds possess less activity. In case of *A.Niger* and *A.Clavatus* all the compounds possess less activity. The results are given in Table-2.

ANTIMALARIAL ACTIVITY

For antimalarial activity, Compounds **8a** and **8b** exhibit good activity closer to reference compound Quinine against *plasmodium falciparum* strain while rest of the compounds possess less activity. The results are given in Table-3.

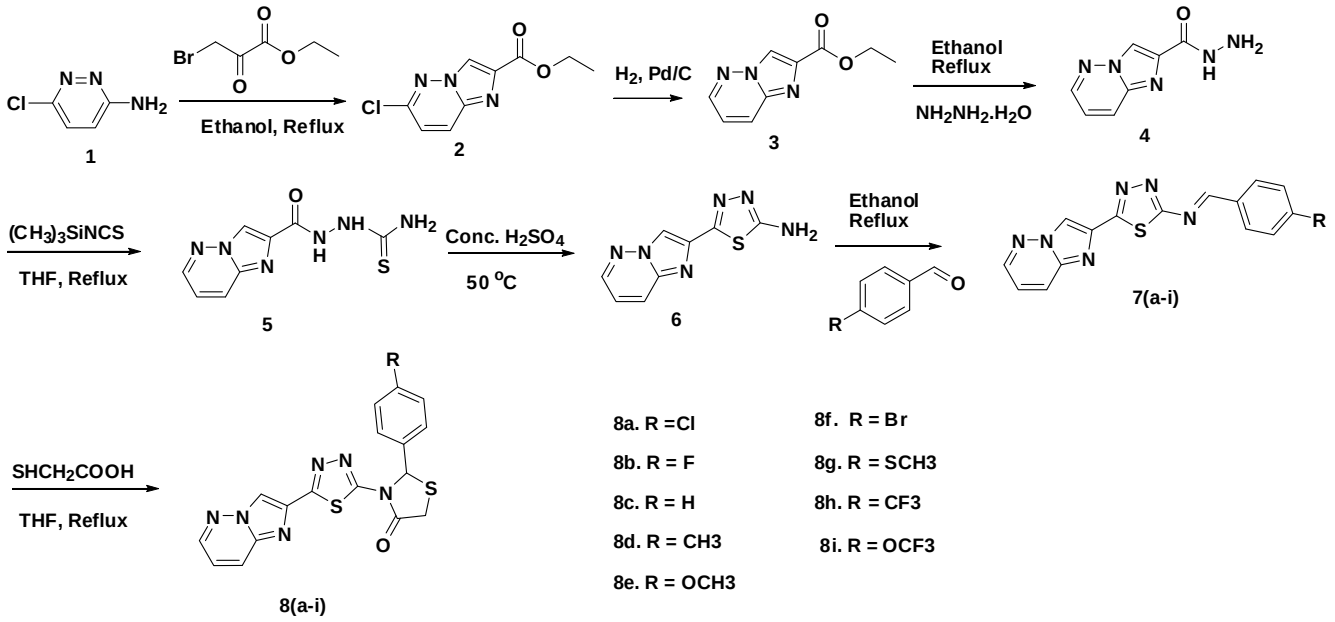
EXPERIMENTAL SECTION

Material and methods

Reagent grade chemicals were used without further purification. All the melting points were taken in open capillaries and are uncorrected. The purity and mass of the synthesized compounds were checked by LCMS . IR spectra (KBr) and (^{1}H &^{13}C) NMR spectra ($CDCl_3$/DMSO-d_6) were recorded on FTIR RXI Perkin-Elmer 1800 spectrophotometer and Bruker DRTX-400 spectrophotometer (400 MHz) using TMS as internal standard, respectively. The chemical shifts are reported as parts per million (ppm). Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respectively.

Chemistry

Ethyl 6-chloroimidazo[1,2-b]pyridazine-2-carboxylate (2): A mixture of 3-amino-6-chloro pyridazine **1** (0.01mole) and ethyl 3-bromo pyruvate (0.012mole) in ethanol (20 ml) was refluxed for 6 hrs. Then it was cooled to room temperature and poured into ice-cold water. The resulting precipitate was filtered, washed several times with water, dried and recrystallized from ethanol.



Scheme: Synthesis of 1,3,4-thiadiazoles derivative

White solid, 83.6% yield, m.p. 140-142 °C. IR(cm-1): 3846.8, 3741.3.4, 3673.8, 3617.5, 2356.5, 1703.9, 1521.9, 1293.0, 1204.3, 1093.0, 1107.8, 1017.8, 840.2, 739.0. ^{1}H-NMR (400MHz, DMSO-d_6): 8.89 (s,1H, Imidazo-H), 8.29 (d, J = 10.0 Hz, 1H, Pyridazine-H), 7.49 (d, J = 9.6 Hz, 1H, Pyridazine-H), 4.33 (q, J = 7.0 Hz, 2H, -$\underline{CH_2}CH_3$), 1.32 (t, J = 7.2 Hz, 3H, -$CH_2\underline{CH_3}$). LC-MS found : m/z 226.1 (M^++1) and calcd for $C_9H_8ClN_3O_2$ is 225.03. Elemental analysis calcd for $C_9H_8ClN_3O_2$: C- 47.91%, H- 3.57%, Cl- 15.71%, N- 18.62%, O- 14.18%; found : C- 47.89%, H- 3.55%, Cl- 15.70%, N- 18.60%, O- 14.16%.

Ethyl imidazo[1,2-b]pyridazine-2-carboxylate (3):A mixture of ethyl 6-chloroimidazo[1,2-b]pyridazine-2-carboxylate **2** (0.01 mole) and 10% Pd/C (10% by wt) in ethanol (30 ml) was stirred at ambient temperature under hydrogen atmosphere for 24 hrs. Then the reaction mixture was filtered through celite bed and evaporated. The crude compound was used as such for next step without any purification.

Offwhite solid, 53.7% yield, m.p. 120-122 °C. IR(cm-1): 3851.6, 3808.7, 3745.6, 3658.5, 3605.7, 3253.8, 2347.1, 1640.4, 1523.3, 1331.5, 1261.7, 1175.1, 1090.4, 935.9, 791.2, 683.5. ^{1}H-NMR (400MHz, DMSO-d_6): 8.84 (s,1H, Imidazo-H), 8.63 (dd, J_1 = 1.2 Hz, J_2 = 4.4 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H, Pyridazine-H), 7.36-7.33 (m, 1H, Pyridazine-H), 4.34 (q, J = 6.8 Hz, 2H, -$\underline{CH_2}CH_3$), 1.32 (t, J = 7.0 Hz, 3H, -$CH_2\underline{CH_3}$). LC-MS found : m/z 192.1 (M^++1) and calcd for $C_9H_9N_3O_2$ is 191.07. Elemental analysis calcd for $C_9H_9N_3O_2$: C- 56.54%, H- 4.74%, N- 21.98%, O- 16.74%; found : C- 56.52%, H- 4.71%, N- 21.95%, O- 16.71%.

Imidazo[1,2-b]pyridazine-2-carbohydrazide (4): A mixture of ethyl imidazo[1,2-b]pyridazine-2-carboxylate **3** (0.01mole) and hydrazine hydrate (0.05 mole) in ethanol (20 ml) was refluxed for 6 hrs. Then it was cooled to room temperature and evaporated. The resulting crude compound was crystallized from ethanol.

Light brown solid, 56.1% yield, m.p. 103-105 °C. IR(cm-1): 3419.2, 3201.2, 2960.2, 2734.1, 1790.2, 1423.5, 1320.3, 1290.1, 951.3. ^{1}H-NMR (400MHz, DMSO-d_6): 9.64 (bs, 1H, NH), 8.63 (s,1H, Imidazo-H), 8.58 (d, J = 2.8 Hz, 1H), 8.15 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.33-7.29 (m, 1H, Pyridazine-H), 4.48 (bs, 2H, NH_2). LC-MS found : m/z 178.2 (M^++1) and calcd for $C_7H_7N_5O$ is 177.07. Elemental analysis calcd for $C_7H_7N_5O$: C- 47.46%, H- 3.98%, N- 39.53%, O- 9.03%; found : C- 47.47%, H- 3.97%, N- 39.51%, O- 9.01%.

2-(imidazo[1,2-b]pyridazine-2-carbonyl)hydrazine carbothioamide (5): A mixture of imidazo[1,2-b]pyridazine-2-carbohydrazide **4** (0.01mole) and trimethyl silyl isothiocyanate (0.012mole) in THF (30 ml) was refluxed for 24 hrs. Then it was cooled to room temperature and evaporated. The resulting crude compound was washed with pentane and recrystallized from ethanol.

Dark brown solid, 35.4% yield, m.p. 111-113 °C. IR(cm-1): 3309.5, 3178.2, 3018.6, 2778.2, 2554.3, 1647.1, 1560.5, 1280.7, 1180.2, 951.2, 841.4, 656.1. ^{1}H-NMR (400MHz, DMSO-d_6): 10.4 (bs, 1H, -$CSNH_2$), 9.37 (bs, 1H, -$CSNH_2$), 8.76 (s,1H, Imidazo-H), 8.62 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H), 8.19 (d, J = 9.6 Hz, 1H, Pyridazine-H), 7.85 (bs, 1H, -CONH), 7.46 (bs, 1H, -NHCS), 7.36-7.32 (m, 1H, Pyridazine-H). LC-MS found : m/z 237.1 (M^++1) and calcd for $C_8H_8N_6OS$ is 236.05. Elemental analysis calcd for $C_8H_8N_6OS$: C- 40.67%, H- 3.41%, N- 35.57%; O- 6.77%; S- 13.57%; found : C- 40.66%, H- 3.39%, N- 35.55%; O- 6.75%; S- 13.56%.

5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-amine (6): A solution of 2-(imidazo[1,2-b]pyridazine-2-carbonyl)hydrazinecarbothioamide **5** (0.01mole) in conc. H_2SO_4 (10ml) was heated at 50 °C for 6 hrs. Then it was cooled to room temperature and poured into ice-cold ammonical water (200 ml) dropwise. The resulting precipitate was filtered, washed several times with water, dried and recrystallized from ethanol.

Offwhite solid, 41.2% yield, m.p. 124-126 °C. IR(cm-1): 3837.7, 3738.7, 3671.7, 3618.2, 2957.7, 2348.5, 1747.1, 1699.2, 1642.2, 1520.7, 1409.2, 1262.9, 1092.7, 1026.5, 804.6, 687.9. ^{1}H-NMR (400MHz, DMSO-d_6): 8.71 (s,1H, Imidazo-H), 8.56 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H), 8.16 (d, J = 8.4Hz, 1H, Pyridazine-H), 7.42 (bs, 1H, -NH_2), 7.31-7.28 (m, 1H, Pyridazine-H). LC-MS found : m/z 219.3 (M^++1) and calcd for $C_8H_6N_6S$ is 218.04. Elemental analysis calcd for $C_8H_6N_6S$: C- 44.03%, H- 2.77%, N- 38.51%, S- 14.69%; found : C- 44.01%, H- 2.75%, N- 38.50%, S- 14.67%.

General procedure for the synthesis of N-(arylidene)- 5-(imidazo [1,2-b] pyridazin-2-yl)-1,3,4-thiadiazol-2-amine (7a-i):

A mixture of 5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-amine **6** (0.01mole) and substituted benzaldehyde (0.01 mole) was refluxed in ethanol for 6-7 hr with a few drops of glacial acetic acid. After completion (monitored by TLC), the reaction mixture was evaporated and and purified by column chromatography by using 100-200 silica gel at 70% ethyl acetate in hexane.

N-(4-chlorobenzylidene)- 5-(imidazo [1,2-b] pyridazin-2-yl)-1,3,4-thiadiazol-2-amine (7a): Pale yellow solid, 39.3% yield, m.p. 132-134 °C. IR(cm-1): 3854.3, 3743.9, 3673.9, 3628.7, 3564.1, 3440.9, 2358.9, 1697.8, 1509.9, 1427.7, 1264.1, 1226.0, 1160.1, 1092.6, 832.7, 785.3, 657.5. ^{1}H-NMR (400MHz, DMSO-d_6): 9.12 (s,1H, N=CH), 9.03 (s,1H, Imidazo-H), 8.64 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.8 Hz, 1H, Pyridazine-H), 8.10 (d, J = 8.8 Hz, 2H, Ar-H), 7.68 (d, J = 8.0Hz, 2H, Ar-H), 7.39-7.35 (m, 1H, Pyridazine-H). LC-MS found : m/z 341.2 (M^++1) and calcd for $C_{15}H_9ClN_6S$ is 340.03. Elemental analysis calcd for $C_{15}H_9ClN_6S$: C- 52.87%, H- 2.66%, Cl- 10.40%, N- 24.66%, S- 9.41%; found : C- 52.85%, H- 2.64%, Cl- 10.38%, N- 24.64%, S- 9.39%.

N-(4-fluorobenzylidene)-5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol-2-amine (7b): Light yellow solid, 21.7% yield, m.p. 114-116 °C. IR(cm-1): 3782.3, 3711.1, 3623.9, 3596.2, 3524.1, 3423.7, 2337.9, 1681.2, 1500.1, 1399.4, 1260.8, 1219.7, 1148.2, 1084.6, 816.9, 770.3, 665.3. ^{1}H-NMR (400MHz, DMSO-d_6): 9.11 (s,1H, N=CH), 9.02 (s,1H, Imidazo-H), 8.64 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.4Hz, 1H, Pyridazine-H), 8.16 (d, J = 7.2 Hz, 2H, Ar-H), 7.46 (d, J = 7.0 Hz, 2H, Ar-H), 7.35-7.29 (m, 1H, Pyridazine-H). LC-MS found : m/z 325.2 (M^++1) and calcd for $C_{15}H_9FN_6S$ is 324.06. Elemental analysis calcd for $C_{15}H_9FN_6S$: C- 55.55%, H- 2.80%, F- 5.86%, N- 25.91%, S- 9.89%; found : C- 55.52%, H- 2.78%, F- 5.84%, N- 25.89%, S- 9.87%.

N-benzylidene-5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-amine (7c): Light yellow solid, 24.1% yield, m.p. 108-110 °C. IR(cm-1): 3813.4, 3734.4, 3665.9, 3610.8, 3561.9, 3470.3, 2345.8, 1697.3, 1531.6, 1440.5, 1291.9, 1249.7, 1182.3, 1098.2, 843.8, 796.1, 640.4. ^{1}H-NMR (400MHz, DMSO-d_6): 9.11 (s,1H, N=CH), 9.02 (s,1H, Imidazo-H), 8.64 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 9.2 Hz, 1H, Pyridazine-H), 8.10-8.08 (m, 2H, Ar-H), 7.70-7.67 (m, 1H, Ar-H), 7.63-7.57 (m, 2H, Ar-H), 7.39-7.35 (m, 1H, Pyridazine-H). LC-MS found : m/z 307.2 (M^++1) and calcd for $C_{15}H_{10}N_6S$ is 306.07. Elemental analysis calcd for $C_{15}H_{10}N_6S$: C- 58.81%, H- 3.29%, N- 27.43%, S- 10.47%; found : C- 58.79%, H- 3.27%, N- 27.42%, S- 10.46%.

5-(imidazo[1,2-b]pyridazin-2-yl) -N-(4-methylbenzylidene) -1,3,4-thiadiazol- 2-amine (7d): Pale yellow solid, 24.5% yield, m.p. 112-114 °C. IR(cm-1): 3834.6, 3761.1, 3656.2, 3629.7, 3586.4, 3493.2, 2347.8, 1691.4, 1540.7, 1454.6, 1274.6, 1261.7, 1178.3, 1102.7, 870.8, 787.3, 671.9. ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, N=CH), 9.02 (s,1H, Imidazo-H), 8.65 (dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.70 (d, J = 7.6Hz, 2H, Ar-H), 7.37-7.33 (m, 1H, Pyridazine-H), 7.30 (d, J = 7.8 Hz, 2H, Ar-H). MS found : m/z 321.3 (M^++1) and calcd for $C_{16}H_{12}N_6S$ is 320.08. Elemental analysis calcd for $C_{16}H_{12}N_6S$: C- 59.98%, H- 3.78%, N- 26.23%, S- 10.01%; found : C- 59.96%, H- 3.76%, N- 26.21%, S- 9.98%.

5-(imidazo[1,2-b]pyridazin-2-yl) -N-(4-methoxybenzylidene) -1,3,4-thiadiazol -2-amine (7e): Yellow solid, 31.5% yield, m.p. 126-128 °C. IR(cm-1): 3828.4, 3753.1, 3671.8, 3634.6, 3591.6, 3501.4, 2368.7, 1698.5, 1549.7, 1461.8, 1283.9, 1269.2, 1184.7, 1119.7, 885.2, 779.3, 680.4. ^{1}H-NMR (400MHz, DMSO-d_6): 9.10 (s,1H, N=CH), 9.02 (s,1H, Imidazo-H), 8.64(dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.25 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.76 (d, J = 8.4 Hz, 2H, Ar-H), 7.37-7.33 (m, 1H, Pyridazine-H), 7.22 (d, J = 8.2 Hz, 2H, Ar-H). LC-MS found : m/z 337.2 (M^++1) and calcd for $C_{16}H_{12}N_6OS$ is 336.08. Elemental analysis calcd for $C_{16}H_{12}N_6OS$: C- 57.13%, H- 3.60%, N- 24.98%, O- 4.76%, S- 9.53%; found : C- 57.11%, H- 3.58%, N- 24.96%, O- 4.74%, S- 9.51%.

N-(4-bromobenzylidene) -5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol -2-amine(7f) : Yellow solid, 46.8% yield, m.p. 142-144 °C. IR(cm-1): 3841.8, 3798.1, 3688.9, 3641.2, 3604.8, 3498.5, 2383.8, 1703.9, 1567.6, 1468.5, 1271.6, 1261.1, 1170.7, 870.2, 760.4, 696.7. ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, N=CH), 9.03 (s,1H, Imidazo-H), 8.64(dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.82 (d, J = 8.0 Hz, 2H, Ar-H), 7.68 (d, J = 7.8 Hz, 2H, Ar-H), 7.39-7.35 (m, 1H, Pyridazine-H). LC-MS found : m/z 385.3 (M^++1) and calcd for $C_{15}H_9BrN_6S$ is 384.0. Elemental analysis calcd for $C_{15}H_9BrN_6S$: C- 46.77%, H- 2.35%, Br- 20.74%, N- 21.81%, S- 8.32%; found : C- 46.75%, H- 2.33%, Br- 20.73%, N- 21.79%, S- 8.30%.

5-(imidazo[1,2-b]pyridazin-2-yl)-N-(4-(methylthio)benzylidene)-1,3,4-thiadiazol-2-amine (7g): Pale yellow solid, 38.4% yield, m.p. 158-160 °C. IR(cm-1): 3901.2, 3820.4, 3698.5, 3678.5, 3621.8, 3510.8, 2391.6, 1712.7, 1571.9, 1479.7, 1287.1, 1276.0, 1181.9, 881.9, 767.6, 705.7. ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, N=CH), 9.03 (s,1H, Imidazo-H), 8.64(dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.65 (d, J = 7.8 Hz, 2H, Ar-H), 7.37-7.33 (m, 1H, Pyridazine-H), 7.30 (d, J = 8.0 Hz, 2H, Ar-H). LC-MS found : m/z 353.3 (M^++1) and calcd for $C_{16}H_{12}N_6S_2$is 352.05. Elemental analysis calcd for $C_{16}H_{12}N_6S_2$: C- 54.53%, H- 3.43%, N- 23.85%, S- 18.20%; found : C- 54.51%, H- 3.41%, N- 23.83%, S- 18.18%.

5-(imidazo[1,2-b]pyridazin-2-yl) -N-(4-(trifluoromethyl)benzylidene) -1,3,4-thiadiazol-2-amine (7h): Light yellow solid, 44.5% yield, m.p. 145-147 ^{o}C. IR(cm-1): 3840.4, 3770.1, 3647.8, 3610.6, 3534.6, 3503.4, 2340.7, 1679.5, 1556.7, 1423.8, 1230.9, 1221.2, 1165.7, 1149.7, 870.2, 749.3, 656.4. ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, N=CH), 9.03 (s,1H, Imidazo-H), 8.64(dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.25 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.92 (d, J = 8.4 Hz, 2H, Ar-H), 7.70 (d, J = 8.2 Hz, 2H, Ar-H), 7.37-7.33 (m, 1H, Pyridazine-H). LC-MS found : m/z 375.2 (M^{+}+1) and calcd for $C_{16}H_9F_3N_6S$ is 374.0. Elemental analysis calcd for $C_{16}H_9F_3N_6S$: C- 51.34%, H- 2.42%, F- 15.23%, N- 22.45%, S- 8.57%; found : C- 51.32%, H- 2.41%, F- 15.21%, N- 22.43%, S- 8.55%.

5-(imidazo[1,2-b]pyridazin-2-yl) -N-(4-(trifluoromethoxy)benzylidene) -1,3,4-thiadiazol-2-amine (7i) : Yellow solid, 35.8% yield, m.p. 156-158 ^{o}C. IR(cm-1): 3873.8, 3778.1, 3645.9, 3634.2, 3611.8, 3467.5, 2367.8, 1723.9, 1532.6, 1440.5, 1289.6, 1249.1, 1149.8, 885.2, 756.4, 667.8. ^{1}H-NMR (400MHz, DMSO-d_6): 9.13 (s,1H, N=CH), 9.03 (s,1H, Imidazo-H), 8.64(dd, J_1 = 1.6 Hz, J_2 = 4.4 Hz, 1H, Pyridazine-H), 8.24 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.95 (d, J = 8.0 Hz, 2H, Ar-H), 7.39-7.35 (m, 1H, Pyridazine-H), 7.10 (d, J = 7.8 Hz, 2H, Ar-H). LC-MS found : m/z 391.3 (M^{+}+1) and calcd for $C_{16}H_9F_3N_6OS$ is 390.0. Elemental analysis calcd for $C_{16}H_9F_3N_6OS$: C- 49.23%, H- 2.32%, F- 14.60%, N- 21.53%, O- 4.10%, S- 8.21%; found : C- 49.22%, H- 2.31%, F- 14.59%, N- 21.51%, O- 4.06%, S- 8.19%.

General procedure for the synthesis of 2-(aryl)-3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl)thiazolidin-4-one (8a-i):

A mixture of N-(arylidene)- 5-(imidazo [1,2-b] pyridazin-2-yl)-1,3,4-thiadiazol-2-amine (Schiff base) **7a-i** (0.01 mole) and thioglycolic acid (0.012 mole) in THF (20 mL) containing a pinch of $ZnCl_2$ was refluxed for 5 hrs. After completion (monitored by TLC), the reaction mixture was cooled to room temperature and poured into ice cold water. The resulting precipitate was filtered, washed several times with water, dried and recrystallized from ethanol.

2-(4-chlorophenyl) -3-(5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol-2-yl)thiazolidin-4-one (8a): Offwhite solid, 74.7% yield, m.p. 172-174 ^{o}C. IR(cm-1): 3954.1, 3802.1, 3750.9, 3654.7, 3604.8, 3521.7, 3401.9, 3289.7, 3096.8, 3038.9, 2971.1, 2912.8, 2854.8, 2737.1, 2621.1, 2561.3, 2508.4, 2352.8, 1753.8, 1699.0, 1538.1, 1439.8, 1311.7, 1146.0, 1091.1, 1031.1, 799.3, 654.7, 624.4. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 7.41 (d, J = 8.4 Hz, 2H, Ar-H), 7.36-7.33 (m, 1H, Pyridazine-H), 6.83 (s, 1H, N-CH-Ar), 4.35 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d6): 171.0, 159.0, 145.3, 139.7, 139.2, 135.5, 132.7, 128.6, 127.8, 125.7, 119.29, 114.7, 62.0, 31.5. LC-MS found : m/z 415.2 (M^{+}+1) and calcd for $C_{17}H_{11}ClN_6OS_2$ is 414.01. Elemental analysis calcd for $C_{17}H_{11}ClN_6OS_2$: C- 49.21%, H- 2.67%, Cl- 8.55%, N- 20.26%, O- 3.86%, S- 15.46%; found : C- 49.20%, H- 2.65%, Cl- 8.53%, N- 20.25%, O- 3.84%, S- 15.44%.

2-(4-fluorophenyl) -3-(5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol -2-yl)thiazolidin-4-one (8b): Pale yellow solid, 70.1% yield, m.p. 158-160 ^{o}C. IR(cm-1): 3939.0, 3883.0, 3831.8, 3733.5, 3622.3, 3575.5, 3479.5, 3390.5, 3314.0, 3193.6, 3126.0, 3006.0, 2891.0, 2750.1, 2653.8, 2372.0, 2312.5, 2230.8, 2132.0, 1656.3, 1652.3, 1634.9, 1618.2, 1514.6, 1427.6, 1358.1, 1317.8, 1195.8, 973.8, 789.0, 736.9, 648.7. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.54-7.50 (m, 2H, Ar-H), 7.36-7.32 (m, 1H, Pyridazine-H), 7.20-7.15 (m, 2H, Ar-H), , 6.83 (s, 1H, N-CH-Ar), 4.37 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 171.0, 162.9, 160.5, 159.0, 156.7, 145.3, 139.2, 136.9, 135.5, 128.1, 125.7, 119.3, 115.5, 115.3, 114.8, 62.0, 28.9. LC-MS found : m/z 399.4 (M^{+}+1) and calcd for $C_{17}H_{11}FN_6OS_2$ is 398.1. Elemental analysis calcd for $C_{17}H_{11}FN_6OS_2$: C- 51.25%, H- 2.78%, F- 4.77%, N- 21.09%, O- 4.02%, S- 16.10%; found : C- 51.24%, H- 2.76%, F- 4.75%, N- 21.07%, O- 4.00%, S- 16.07%.

3-(5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol-2-yl) -2-phenylthiazolidin-4-one (8c): Yellow solid, 70.1% yield, m.p. 158-160 ^{o}C. IR(cm-1): 3903.0, 3880.1, 3811.9, 3754.9, 3642.8, 3580.8, 3494.7, 3401.8, 3324.0, 3160.6, 3130.1, 2997.1, 2903.4, 2761.6, 2676.0, 2384.0, 2334.5, 2243.7, 2139.0, 1677.3, 1652.3, 1640.9, 1530.6, 1442.9, 1370.1, 1340.8, 1170.7, 954.8, 791.8, 740.3, 660.5. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 8.8 Hz, 1H, Pyridazine-H), 7.43-7.41 (m, 2H, Ar-H), 7.37-7.34 (m, 2H, Ar-H), 7.33-7.27 (m, 1H, Pyridazine-H), 6.83 (s, 1H, N-CH-Ar), 4.34 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.8 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 171.0, 160.3, 144.5, 139.1, 139.2, 136.8, 130.7, 127.5, 126.3, 125.7, 122.2, 116.1, 62.2, 30.5. LC-MS found : m/z 381.4 (M^{+}+1) and calcd for $C_{17}H_{12}N_6OS_2$ is 380.1. Elemental analysis calcd for $C_{17}H_{12}N_6OS_2$: C- 53.67%, H- 3.18%, N- 22.09%, O- 4.21%, S- 16.86%; found : C- 53.66%, H- 3.16%, N- 22.07%, O- 4.19%, S- 16.84%.

3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl)-2-p-tolylthiazolidin-4-one (8d): White solid, 74.7% yield, m.p. 172-174 ^{o}C. IR(cm-1): 3872.2, 3839.2, 3807.9, 3780.3, 3672.4, 3591.8, 3467.3, 3378.9, 3310.3, 3131.8, 3111.3, 2988.3, 2915.4, 2710.2, 2630.3, 2360.0, 2356.3, 2250.7, 2203.2, 1677.3, 1640.3, 1618.6, 1521.6, 1414.9, 1351.4, 1327.4, 1140.7, 933.5, 777.8, 760.3, 635.5. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.60 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.36-7.33 (m, 1H, Pyridazine-H), 7.24 (d, J = 8.4 Hz, 2H, Ar-H), 7.20 (d, J = 8.4 Hz, 2H, Ar-H), 6.83 (s, 1H, N-CH-Ar), 4.36 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.07 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 171.2, 159.1, 147.4, 140.1, 138.2, 136.1, 129.1, 128.5, 125.8, 123.4, 111.1, 62.7, 31.1, 19.2. LC-MS found : m/z 395.4 (M^{+}+1) and calcd for $C_{18}H_{14}N_6OS_2$ is 394.1. Elemental analysis calcd for $C_{18}H_{14}N_6OS_2$: C- 54.81%, H- 3.58%, N- 21.30%, O-

4.06%, S- 16.26%; found : C- 54.79%, H- 3.56%, N- 21.29%, O- 4.05%, S- 16.24%

3-(5-(imidazo [1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol-2-yl)-2-(4-methoxyphenyl) thiazoli-din-4-one (8e): Offwhite solid, 74.7% yield, m.p. 178-180 ^{o}C. IR(cm-1): 3891.1, 3840.2, 3796.9, 3768.4, 3683.6, 3600.6, 3480.7, 3350.8, 3296.7, 3120.4, 3100.3, 2970.5, 2900.4, 2696.1, 2629.6, 2339.0, 2311.3, 2271.7, 2196.6, 1660.3, 1630.3, 1605.6, 1510.8, 1405.1, 1375.8, 1345.4, 1121.7, 911.8, 750.8, 720.3, 610.5. ^{1}H-NMR (400MHz, DMSO-d_6): 8.89 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.76 (d, J = 8.8 Hz, 2H, Ar-H), 7.36-7.33 (m, 1H, Pyridazine-H), 6.88 (d, J = 8.4 Hz, 2H, Ar-H), 6.84 (s, 1H, N-CH-Ar), 4.36 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 170.1, 160.4, 149.4, 143.2, 140.2, 138.1, 132.8, 127.5, 126.9, 123.4, 115.9, 62.0, 50.3, 29.9. LC-MS found : m/z 411.4 (M^{+}+1) and calcd for $C_{18}H_{14}N_6O_2S_2$ is 410.0. Elemental analysis calcd for $C_{18}H_{14}N_6O_2S_2$: C- 52.67%, H- 3.44%, N- 20.47%, O- 7.80%, S- 15.62%; found : C- 52.65%, H- 3.43%, N- 20.46%, O- 7.78%, S- 15.60%.

2-(4-bromophenyl) -3-(5-(imidazo[1,2-b]pyridazin-2-yl) -1,3,4-thiadiazol -2-yl)thiazolidin-4-one (8f): Pale yellow solid, 81.2% yield, m.p. 192-194 ^{o}C. IR(cm-1): 3912.5, 3780.1, 3720.8, 3640.9, 3598.8, 3511.7, 3391.9, 3231.7, 3070.8, 3012.9, 2948.1, 2901.6, 2823.9, 2712.3, 2611.9, 2526.4, 2498.4, 2321.8, 1700.8, 1659.0, 1517.1, 1430.8, 1305.7, 1110.0, 1067.6, 1045.4, 745.3, 613.7, 604.4. ^{1}H-NMR (400MHz, DMSO-d_6): 8.88 (s,1H, Imidazo-H), 8.60 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.72 (d, J = 8.4 Hz, 2H, Ar-H), 7.37-7.33 (m, 1H, Pyridazine-H), 7.20 (d, J = 8.8 Hz, 2H, Ar-H), , 6.83 (s, 1H, N-CH-Ar), 4.37 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 170.1, 160.1, 150.4, 147.1, 143.3, 140.1, 136.8, 131.9, 129.9, 124.4, 120.9, 62.1, 30.3. LC-MS found : m/z 459.1 (M^{+}+1) and calcd for $C_{17}H_{11}BrN_6OS_2$ is 457.9. Elemental analysis calcd for $C_{17}H_{11}BrN_6OS_2$: C- 44.45%, H- 2.41%, Br- 17.40%, N- 18.30%, O- 3.48%, S- 13.96%; found : C- 44.43%, H- 2.39%, Br- 17.38%, N- 18.29%, O- 3.46%, S- 13.97%.

3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl)-2-(4-(methylthio)phenyl) thiazolidin-4-one (8g): Offwhite solid, 74.7% yield, m.p. 178-180 ^{o}C. IR(cm-1): 3934.0, 3865.4, 3829.4, 3721.5, 3602.3, 3569.3, 3431.5, 3390.5, 3301.0, 3163.6, 3112.0, 2996.0, 2877.0, 2712.1, 2667.8, 2356.0, 2322.5, 2207.8, 2147.9, 1679.3, 1660.3, 1650.9, 1648.2, 1540.6, 1449.8, 1334.1, 1301.8, 1199.8, 960.8, 780.0, 710.9, 623.7. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.60 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.36-7.33 (m, 1H, Pyridazine-H), 7.10 (d, J = 8.8 Hz, 2H, Ar-H), 6.90 (d, J = 8.4 Hz, 2H, Ar-H), 6.84 (s, 1H, N-CH-Ar), 4.36 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 170.8, 160.7, 147.2, 145.1, 139.2, 136.1, 134.8, 131.5, 129.9, 126.1, 122.1, 62.1, 30.1, 22.1. LC-MS found : m/z 427.3 (M^{+}+1) and calcd for $C_{18}H_{14}N_6OS_3$ is 426.0. Elemental analysis calcd for $C_{18}H_{14}N_6OS_3$: C- 50.69%, H- 3.31%, N- 19.70%, O- 3.75%, S- 22.55%; found : C- 50.67%, H- 3.29%, N- 19.68%, O- 3.73%, S- 22.56%.

3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl) -2-(4-(trifluoromethyl) phenyl) thiazolidin -4-one (8h): Light yellow solid, 62.7% yield, m.p. 187-189 ^{o}C. IR(cm-1): 3976.1, 3823.1, 3739.9, 3667.8, 3621.8, 3513.7, 3387.4, 3291.7, 3067.8, 3018.9, 2947.1, 2901.8, 2834.8, 2715.1, 2645.2, 2546.3, 2500.4, 2327.8, 1738.8, 1700.4, 1516.1, 1467.8, 1302.5, 1135.0, 1087.5, 1040.3, 780.3, 667.7, 630.7. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.49 (d, J = 8.4 Hz, 2H, Ar-H), 7.36-7.33 (m, 1H, Pyridazine-H), 7.03 (d, J = 8.4 Hz, 2H, Ar-H), 6.83 (s, 1H, N-CH-Ar), 4.36 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.08 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 171.2, 161.0, 145.3, 137.7, 136.2, 134.5, 130.7, 128.6, 127.8, 125.7, 120.2, 114.7, 62.0, 31.5. LC-MS found : m/z 449.3 (M^{+}+1) and calcd for $C_{18}H_{11}F_3N_6OS_2$ is 448.4. Elemental analysis calcd for $C_{18}H_{11}F_3N_6OS_2$: C- 48.21%, H- 2.47%, F- 12.71%, N- 18.74%, O- 3.57%, S- 14.30%; found : C- 48.20%, H- 2.45%, F- 12.69%, N- 18.73%, O- 3.55%, S- 14.29%.

3-(5-(imidazo[1,2-b]pyridazin-2-yl)-1,3,4-thiadiazol-2-yl)-2-(4-(trifluoromethoxy) phenyl) thiazolidin-4-onem (8i): Offwhite solid, 61.1% yield, m.p. 149-151 ^{o}C. IR(cm-1): 3978.0, 3856.0, 3810.8, 3744.5, 3656.3, 3523.5, 3424.5, 3347.5, 3320.0, 3180.6, 3164.3, 2991.7, 2878.3, 2770.7, 2649.8, 2350.7, 2309.5, 2243.8, 2120.0, 1660.3, 1642.3, 1634.9, 1618.2, 1549.6, 1427.6, 1312.1, 1303.8, 1189.8, 989.8, 775.0, 729.9, 619.7. ^{1}H-NMR (400MHz, DMSO-d_6): 8.87 (s,1H, Imidazo-H), 8.61 (d, J = 3.2 Hz, 1H, Pyridazine-H), 8.22 (d, J = 9.2 Hz, 1H, Pyridazine-H), 7.82 (d, J = 8.4 Hz, 2H, Ar-H), 7.36-7.32 (m, 1H, Pyridazine-H), 7.25 (d, J = 8.4 Hz, 2H, Ar-H), , 6.83 (s, 1H, N-CH-Ar), 4.36 (d, J = 16.8 Hz, 1H, SCH_2CO), 4.07 (d, J = 16.4 Hz, 1H, SCH_2CO). ^{13}C NMR (DMSO-d_6): 171.3, 161.9, 159.0, 150.7, 141.3, 139.2, 135.9, 132.5, 127.1, 124.7, 119.3, 115.3, 114.8, 62.7, 27.9. LC-MS found : m/z 465.4 (M^{+}+1) and calcd for $C_{18}H_{11}F_3N_6O_2S_2$ is 464.1. Elemental analysis calcd for $C_{18}H_{11}F_3N_6O_2S_2$: C- 46.55%, H- 2.39%, F- 12.27%, N- 18.09%, O- 6.89%, S- 13.81%; found : C- 46.53%, H- 2.38%, F- 12.25%, N- 18.07%, O- 6.87%, S- 13.79%.

IN VITRO ANTIMICROBIAL ASSAY

All the synthesized compounds were tested against two gram positive bacteria (*Staphylococcus aureus*, *Streptococcus Pyogenes*) and two gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) using micro broth dilution method[24-27] for the determination of minimal inhibition concentration. For the antifungal activity the common standard strains that were used, are *C. Albicans*, *A. Niger* and *A. Clavatus*. Muller Hinton broth (Microcare laboratory & Tuberculosis Research Centre, Surat-3, India) was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum Size for Test Strain was adjusted to 10^8 Cfu [Colony Forming Unit] per milliliter by comparing the turbidity. DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains. Serial dilutions were preparedin primary and secondary screening. In primary screening 1000 µg/ml, 500 µg/ml, and 250 µg/ml concentrations of the

synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The highest dilution showing at least 99 % inhibition zone is taken as MIC (minimum inhibitory concentration). The test mixture should contain 10^8 organism/ml. Standard drugs Ampicillin and Chloramphenicol were used as antibacterial for comparison. Standard drugs Nystatin and Greseofulvin were used as antifungal for comparison.

Table 1: Antibacterial activity (MIC in µg/ml)

Compound	S. aureus	S. pyogenus	E. coli	P. aeruginosa
8a	125	500	62.5	100
8b	125	250	62.5	100
8c	500	500	100	125
8d	500	125	125	100
8e	125	500	200	500
8f	100	100	250	125
8g	125	125	250	125
8h	100	500	125	500
8i	250	500	250	250
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

Table 2: Antifungal activity (MIC in µg/ml)

Compound	C.Albicans	A.Niger	A.Clavatus
8a	250	500	>1000
8b	250	>1000	>1000
8c	500	500	500
8d	250	1000	500
8e	500	500	500
8f	500	500	500
8g	1000	500	250
8h	500	250	500
8i	500	250	250
Nystatin	100	100	100
Greseofulvin	500	100	100

Table 3: Antimalarial activity

Compound	Mean IC50 (µg/ml)
8a	0.67
8b	0.56
8c	0.80
8d	0.95
8e	1.15
8f	0.97
8g	1.10
8h	1.25
8i	0.91
Quinine	0.268

IN VITRO ANTIMALARIAL ASSAY

The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol reference. The cultures of *Plasmodium falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *Plasmodium falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µ l of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformally maintained with 50% RBCs (O+). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 µ g/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37 ^{o}C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was taken as the reference drug.

CONCLUSION

In conclusion, we have designed and synthesized some new 1,3,4-thiadiazoles derivatives incorporating imidazo [1,2-b] pyridazine and thiazolidinone moieties, and characterized through elemental and spectral analysis. These derivatives were evaluated for antimicrobial and antimalarial activity. It can be concluded from antibacterial screening (Table-1) that compound **8a** and **8b** were found to be active against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively compared to the rest of the compounds. However, the activities of the tested compounds are much less than those of standard agents used. The compounds **8a** and **8b** also showed good antimalarial activity but not superior to the standard.

Further synthetic modification is required to enhance the potency of 1,3,4-thiadiazoles derivatives by changing molecular configuration, which is in progress at our laboratory. The present study throws light on the identification of this new structural class as antimicrobials and antimalarial, which can be of interest for further detailed preclinical investigations.

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