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Synthesis of novel pyrido[2,3-*d*]pyrimidine derivatives with pharmacological properties

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In search for new anti-microbial and anti-mycobacterial agents with novel mechanism of action and enhanced biological profile, a combinatorial library of Schiff bases (**7a-e**), 2,3-disubstituted-4-thiazolidinone (**8a-e**) and 2,3-disubstituted-5-methyl-4-thiazolidinone (**9a-e**) derivatives have been synthesized as new pharmacophores under standard Vilsmeier-Haack reaction conditions. The structures of all the newly synthesised compounds have been confirmed from their FT-IR, ^{1}H and ^{13}C NMR, mass spectral as well as elemental analysis data. All the newly synthesized compounds have been screened for non-automated *in vitro* antimicrobial and anti-mycobacterial activity against selected pathogens. Some of the newly synthesized compounds exhibit excellent antimicrobial activity and found to be the most proficient members of the series compared to standard drugs and hold promise as future drugs.

Keywords: 4-Amino-2,6-dimetoxypyrimidine, Schiff base, 4-Thiazolidinone, Vilsmeier-Haack reaction, Antimicrobial activity

The number of serious infectious diseases caused by multidrug-resistant bacteria has reached an alarming level in many countries around the world, in present years there has been a growing interest in the use of synthetic substance against infectious diseases[1]. The development of resistance to current antibacterial therapy continues to drive the search for more effective agents. Bacteria are commonly responsible for many diseases, which were considered until recently to be resistant to chemotherapy moreover there are numbers of regimen agents available to treat such kind of diseases caused by the bacteria described herewith[2]. Research on new substances possessing antibacterial activity has great attention owing to the unremitting raise in bacterial fight[3]. In the recent period, some tubercular strains of *Mycobacterium tuberculosis* cause MDR-Tuberculosis (TB) and extensively drug-resistant XDR-TB which generally affects the lungs[4]. It has been reported that Large numbers of Schiff bases have shown to exhibit a wide range of biological activities, including anti-bacterial[5], fungicidal[6], anti-tubercular[7], anti-HIV[8], antiviral[9], anti-inflammatory[10], anticancer[11], and anti-fertility-activities[12]. On the other hand, co-ordination compounds with heterocyclic Schiff base has attracted much attention of the chemist in current years to find applications as potential drugs[13], due to the presence of multifunctional groups[14]. Schiff bases contain donor sites with various coordination abilities[15]. This nature of the Schiff bases has attracted our attention and vivid in elucidating the structure of this derivatives[16].

There are various biologically active molecules which contain a variety of heteroatoms such as nitrogen, sulphur and oxygen, always drawn the attention of chemist over the years essentially because of their biological magnitude. Thiazolidinones are thiazolidine derivatives and have an atom of sulphur at position 1, an atom of nitrogen at position 3 and a carbonyl group at position 2, 4, or 5. However, its derivatives belong to the most frequently studied moieties and its presence in penicillin was the first recognition of its occurrence in nature[17]. various 4-thiazolidinones contain fascinated considerable attention as they are also capable with a wide range of pharmaceutical activities[18,19], including anticonvulsant[20,21], Antibacterial[22], Anti-tubercular[23], and antiviral[24]. Some thiazolidinones were recently reported as novel inhibitors of mycobacterial rhamnose synthetic enzymes. This new approach is believed to be discriminating as rhamnose is not found in humans, but is important for mycobacterial cell wall synthesis in animals[25]. Looking to the medicinal magnitude of Schiff bases and 4-thiazolidinone, we report here the synthesis of new class of heterocyclic molecules in which all of these moieties are present moreover try to develop potential bioactive molecules.

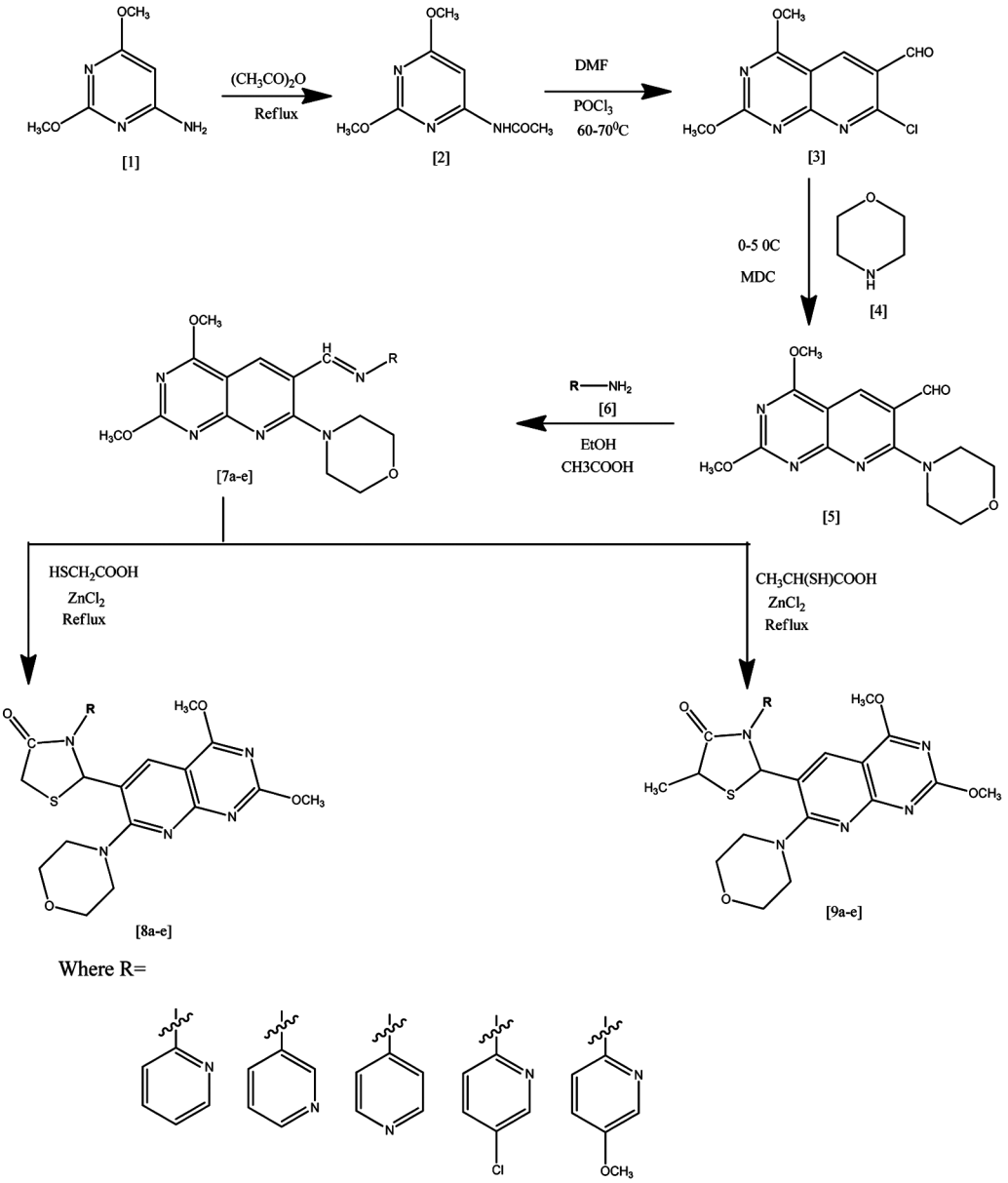
Hence, for the purpose of obtaining new and more potent antitubercular compounds that can improve the current chemotherapeutic antituberculosis treatment, we have synthesized Schiff bases and convert them into its derivatives and tested for antimicrobial and antitubercular activity.

Results and Discussion

Chemistry

The strategy acquired for the synthesis of **7a-e**, **8a-e** and **9a-e** is depicted in Scheme 1. The attack of sulphur nucleophile on imine carbon followed by intramolecular cyclization with elimination of water gives 2,3-disubstituted-4-thiazolidinones **(8a-e)** and 2,3-disubstituted-5-methyl-4-thiazolidinones **(9a-e)** derivatives which were confirmed by spectral analysis.

The formation of titled compound was confirmed by their FT-IR, ^{1}H and ^{13}C NMR, mass spectra as well as elemental analysis. As an example, in the IR spectrum of compound **7c**, characteristic is the N=CH stretching vibration, which appear as an intense band at 1622 cm^{-1}. There was no absorption in between 3300-3400 cm^{-1} which confirmed that free amino



Scheme 1 — Scheme for the synthesis of **7a-e**, **8a-e** and **9a-e**

group of pyridine ring is converted into a proposed imine. The structural element characteristic for the pyrimidine nucleus, namely; the stretching vibration band for the C=N stretching observed at 1551 cm^{-1} respectively. Several bands appeared at 1475 and 3078 cm^{-1} are due to the stretching of C=C and C-H vibrations of aromatic ring moreover C-O-C linkage and C-N stretching observed at 1135 and 1372 in the presence of morpholine ring. Also, electron withdrawing group $–OCH_3$ stretching vibration band observed at 1184 cm^{-1}. The ^{1}H NMR spectrum of compound **7c** did not only show the absence of NH_2 protons of pyridine ring as singlet signal at between δ 5-6 but exerted a singlet at higher field at δ 8.8 for -CH=N proton of the imine group. There was emphasized signal as triplet for the morpholine ring contain protons core at δ 3.6. The two singlets of methoxy group protons are observed at δ 3.8 and remaining protons resonated in the region at δ 7.12-8.12 as multiplet signal. Finally, the ^{13}C NMR spectrum of the product **7c** was recorded in DMSO-d_6 and the spectral signals were in good agreement with the proposed structure. The chemical shifts for the carbon atoms of imine group is observed at δ 160.4 while the carbon atoms of OCH_3 and C=N functionality of pyrimidine scaffold observed at δ 54.6 and 161.1 respectively. The signals for aromatic carbons appeared between δ 100.6-161.1 in the ^{13}C spectrum.

The strong absorption band observed at between 1650-1750 and 600-700 cm^{-1} for the presence of cyclic amido C=O group and C-S-C linkage of thiazolidine unit in both **8d** and **9e**. There was no absorption in the region of 1605-1621 cm^{-1} which 54.5 signifying the disappearance of imine group in this structure. Moreover, the compound **9e** showed a strong absorption band at 1359 cm^{-1} due to the presence of the CH_3 group attached on the **C-5** position of thiazolidine ring which also confirmed the cyclocondensation of imine A broad stretching band for the C=N functionality and OCH_3 group of pyrimidine ring is observed at between 1495-1635 cm^{-1} and 1100-1200 cm^{-1} respectively. The ^{1}H NMR spectrum of compound **8d** showed two doublet of doublet at δ 3.9 (J = 4.6 Hz) and 4.6 (J = 4.9 Hz) due to CHX and CHy protons of active methylene group of the thiazolidine ring. The ^{1}H NMR spectrum of compound **9e** showed diagnostic peaks at δ 3.6 and 4.1 (J = 7.1 Hz) as singlet and quartet due to, Ar-CH and $CH\text{-}CH_3$ proton of the thiazolidine ring system. Finally, the ^{13}C NMR spectra of the cyclised product **8d** and **9e** were recorded in DMSO-d_6 and the spectral signals were in good agreement with the proposed structure. In the ^{13}C NMR spectrum of compound **8d**, the shielded signal at δ 66.4 and 49.6 were assigned to the $–CH_2$ carbon of morpholine ring and methylene carbon of thiazolidine ring whereas in the^{13}C NMR spectrum of compound **9e**, the characteristic signal observed at δ 20.5 for methyl carbon along with the peaks at δ 42.4 and 65.4 for ring carbons. Moreover, the most deshielded signal that appeared at between δ 170.8-173.9 was assigned to the carbonyl group of thiazolidine ring and the signals for aromatic carbons appeared at between δ 100.7-163.7 in the ^{13}C spectrum of both compounds **8d** and **9e**. Further, mass spectra of all the title compounds showed molecular ion peak M^+ corresponding to their mass which is also in agreement with its proposed structure. The obtained elemental analysis values are in good agreement with theoretical data.

Experimental Section

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates (GF 254) using UV light as visualizing agent. Column chromatography was performed with silica gel mesh size 60-120. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer at ambient temperature, using DMSO-d_6 as solvents. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference and J values are given in Hz. Mass spectrometric data were recorded at Waters Micromass Q-Tof Micro. Elemental analysis was done with Thermo Scientific (Flash 2000) analyzer. Ethylacetate:Hexane and chloroform: methanol were the adopted solvent systems.

Preparation of N-(2, 6-dimethoxypyrimidin-4-yl)acetamide, 2

To (0.01 mole) of compound **(1)** (10 mL) of Acetic anhydride and 1-2 drop of acetic acid was added and the mixture was heated under reflux for 6 h. The reaction mixture was poured into crushed ice (200 g) with stirring. The separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound **(2)** in yields of 82%. White

solid. m.p.107°C. Yield 82%. Anal. Calcd for $C_8H_{11}N_3O_3$: C, 48.73; H, 5.62; N, 21.31%. Found C, 48.76; H, 5.65; N, 21.35%. IR (KBr): 3076 (C-H str., aromatic), 1593 (C=C str., aromatic), 1591 (C=N str., pyrimidine ring), 1370 (CH_3 str.), 1681 (C=O str.), 3179 (-NH str. 2°amine), 1177 cm^{-1} (-OCH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, 2°amide), 2.54 (s, 3H, CH_3), 3.64 (s, 3H,OCH_3), 3.73 (s, 3H, OCH_3), 8.31 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 53.8 (OCH_3), 53.6 (OCH_3), 87.8 (C), 165.0 (C), 169.1 (C), 152.5 (C), 166.5 (C=O), 23.9 (CH_3); MS: *m/z* 198.08 (M^+ +1).

Preparation of 7-chloro-2, 4–dimethoxy pyrido [2,3-*d*]pyrimidine-6-carbaldehyde, 3

A mixture of compound **(2)** (0.810 mg, 5 m mol) in DMF (4.0 mL, 50 m mol), $POCl_3$ (0.5 mL, 5 m mol) was added at RT, producing a semi-solid mass. A clear solution appeared after stirring for 4 h at RT. It was further stirred for 6 h. The reaction mixture was poured into crushed ice (200 g) with stirring. The separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound **(3)** in yields of 77%. Light-yellow. m.p.115°C. Yield 77%. Anal. Calcd for $C_{10}H_8ClN_3O$: C, 47.35; H, 3.18; N, 16.57%. Found C, 47.32; H, 3.15; N, 16.54%. IR (KBr): 3076 (C-H str., aromatic), 1593 (C=C str., aromatic), 1591 (C=N str.), 1681 (C=O str. aldehydes), 2785 – 2845 (C-H str. Aldehydes), 709 (C-Cl str.), 1177 cm^{-1} (-OCH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, -CHO), 3.61 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 8.33 (s, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.2 (OCH_3), 54.1 (OCH_3), 162.9 (C), 161.2 (C), 151.5 (C), 131.8 (C), 139.2 (CH), 188.8 (CH), 158.8 (C), 100.6 (C); MS: *m/z* 254.2 (M^+ +1).

Preparation of 2, 4–dimethoxy-7-morpholino pyrido[2,3-*d*]pyrimidine-6-carbaldehyde, 5

A solution of morpholine **(4)** (1.75 g, 20 m mol) in 10 mL of dichloromethane was gradually added under stirring to an ice-cooled mixture of compound **(3).** After stirring for 30 min. at 0-5°C the mixture was washed with 3×10 mL of water in order to remove unreacted morpholine and its salt. The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The dry, flake-like residue were recrystallized from 1, 4-dioxane. The progress of reaction was monitored by TLC using chloroform: methanol (9:1) as eluent. The crude product was purified by crystallization from acetone to get the title compound **(5)** in yields of 70%. IR (KBr): 3078 (C-H str., aromatic), 1598 (C=C str., aromatic), 1590 (C=N str.), 1681 (C=O str. aldehydes), 2785 – 2845 (C-H str. Aldehyeds) 1136 (C-O-C str., morpholine ring), 1374 cm^{-1} (C-N str., morpholine ring); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, -CHO), 3.62 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 8.34 (s, 1H, Ar-H), 2.36 (t, 4H,CH_2 morpholine ring),2.42 (t, 4H, morpholine ring); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.1 (OCH_3), 54.3 (OCH_3), 160.9 (C), 163.1 (C), 159.3 (C), 100.5 (C), 138.4 (CH), 190.8 (CH), 116.4 (C), 159.4 (C), 48.6 (CH_2), 48.6 (CH_2), 66.1 (CH_2), 66.1 (CH_2); MS: *m/z* 305.11 (M^+ +1). Light-yellow, M.P:102°C. Yield 70%. Anal. Calcd for $C_{14}H_{16}N_4O_4$: C, 55.26; H, 5.30; N, 18.41%. Found C, 55.23; H, 5.33; N, 18.44%. IR (KBr): 3078 (C-H str., aromatic), 1598 (C=C str., aromatic), 1590 (C=N str.), 1681 (C=O str. aldehydes), 2785 – 2845 (C-H str. Aldehydes) 1136 (C-O-C str., morpholine ring), 1374 (C-N str., morpholine ring); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H, -CHO), 3.62 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 8.34 (s, 1H, Ar-H), 2.36 (t, 4H,CH_2 morpholine ring),2.42 (t, 4H, morpholine ring); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.1 (OCH_3), 54.3 (OCH_3), 160.9 (C), 163.1 (C), 159.3 (C), 100.5 (C), 138.4 (CH), 190.8 (CH), 116.4 (C), 159.4 (C), 48.6 (CH_2), 48.6 (CH_2), 66.1 (CH_2), 66.1 (CH_2); MS: *m/z* 305.11 (M^+ +1).

General procedure for the preparation of substituted Schiff bases, 7a-e

A solution of **(4)** (2.0 g, 20 m mol) in 20 mL ethanol was added to equimolecular quantities of an amine **(6)** add 1-2 drop of Acetic acid. The reaction mixture was refluxed for 3 h at 60-70°C The separated solid was filtered off and washed thoroughly with water. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The solid product obtained was filtered, washed with water and dried. The crude product was purified by crystallization from acetone to get the title compound **(7).**

***(E)*-N-((2, 4-Dimethoxy-7-morpholinopyrido[2,3-*d*] pyrimidin-6-yl) methylene) pyridin-2-amine, 7a**: Orange solid; m.p.192°C. Yield 76%. Anal. Calcd For $C_{19}H_{20}N_6O_3$: C, 59.99; H, 5.33; N, 22.07%. Found C,

59.96; H, 5.33; N, 22.07%. IR (KBr): 3072 (C-H str., aromatic), 1478 (C=C str., aromatic), 1560 (C=N str.), 1627 (C=N str., imine), 1187 (OCH_3 str.), 1137 (C-O-C str., morpholine ring), 1370 cm^{-1} (C-N str., morpholine ring); ^1H NMR (400 MHz, DMSO-d_6): δ 3.68 (t, 4H, CH_2 morpholine), 3.67 (t, 4H, CH_2 morpholine), 3.87 (s, 3H, -OCH_3), 3.86 (s, 3H, -OCH_3), 6.06 (s, 1H, Ar-H), 8.84 (s, 1H, CH=N), 7.13-8.12 (m, 4H, Ar-H),; ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.6 (OCH_3), 54.4 (OCH_3), 137.8 (CH), 160.4 (C=N, imine), 161.3 (C), 152.8 (C), 161.2 (C), 161.6 (C), 161.4 (C), 48.5 (CH_2), 48.5 (CH_2), 66.4 (CH_2), 66.4 (CH_2), 118.5 (CH), 100.6 (C), 111.4 (C), 138.3 (CH), 119.4 (CH), 145.3 (CH); MS: *m/z* 381.7 (M^+ +1).

***(E)*-N-((2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6yl)methylene) pyridin-3-amine, 7b**: Orange solid; m.p.: 189°C. Yield 73%. Anal. Calcd for $C_{19}H_{20}N_6O_3$: C, 59.99; H, 5.33; N, 22.07%. Found C, 59.95; H, 5.35; N, 22.06%. IR (KBr): 3078 (C-H str., aromatic), 1475 (C=C str., aromatic), 1557 (C=N str.), 1628 (C=N str., imine), 1184 (OCH_3 str.), 1135 (C-O-C str., morpholine ring), 1372 cm^{-1} (C-N str., morpholine ring);^1H NMR (400 MHz, DMSO-d_6): δ 3.65 (t, 4H, CH_2 morpholine), 3.67 (t, 4H, CH_2 morpholine), 3.83 (s, 3H, -OCH_3), 3.85 (s, 3H, -OCH_3), 6.04 (s, 1H, Ar-H), 8.85 (s, 1H, CH=N), 7.11-8.11 (m, 4H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.6 (OCH_3), 54.4 (OCH_3), 137.8 (CH), 160.4 (C=N, imine), 161.3 (C), 152.8 (C), 161.2 (C), 161.6 (C), 161.4 (C), 48.5 (CH_2), 48.5 (CH_2), 66.4 (CH_2), 66.4 (CH_2), 118.5 (CH), 100.6 (C), 111.4 (C), 138.3 (CH), 119.4 (CH), 145.3 (CH); MS: *m/z* 381.7 (M^+ +1).

***(E)*-N-((2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)methylene) pyridin-4-amine, 7c**: Orange solid; m.p.: 195 °C. Yield 78%. Anal. Calcd for $C_{19}H_{20}N_6O_3$: C, 59.99; H, 5.33; N, 22.07%. Found C, 59.94; H, 5.27; N, 22.04%. IR (KBr): 3061 (C-H str., aromatic), 1464 (C=C str., aromatic), 1551 (C=N str.), 1618 (C=N str., imine), 1196 (OCH_3 str.), 1121 (C-O-C str., morpholine ring), 1377 cm^{-1} (C-N str., morpholine ring);^1H NMR (400 MHz, DMSO-d_6): δ 3.67 (t, 4H, CH_2 morpholine), 3.76 (t, 4H, CH_2 morpholine), 3.80 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 6.82 (s, 1H, Ar-H), 8.89 (s, 1H, CH=N), 7.18 (d, J=7.30 Hz, 2H, Ar-H), 7.26 (d, J=7.36 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.6 (OCH_3), 54.4 (OCH_3), 137.8 (CH), 160.4 (C=N, imine), 161.3 (C), 152.8 (C), 161.2 (C), 161.6 (C), 161.4 (C), 48.5 (CH_2), 48.5 (CH_2), 66.4 (CH_2), 66.4 (CH_2), 118.5 (CH), 100.6 (C), 111.4 (C), 138.3 (CH), 119.4 (CH), 145.3 (CH); MS: *m/z* 381.7 (M^+ +1).

***(E)*-5-Chloro-N((2,4-dimethoxy-7-morpholinopyrido [2,3-*d*]pyrimidin-6-yl) methylene) pyridin-2-amine, 7d**: Orange solid; m.p.: 185 °C. Yield 70%. Anal. Calcd for $C_{19}H_{19}ClN_6O_3$: C, 55.01; H, 4.62; N, 20.26%. Found C, 55.04; H, 4.65; N, 20.29%. IR (KBr): 3075 (C-H str., aromatic), 1470 (C=C str., aromatic), 1559 (C=N str.), 1629 (C=N str., imine), 1190 (OCH_3 str.), 1138 (C-O-C str., morpholine ring), 1373 (C-N str., morpholine ring) 658 cm^{-1} (C-Cl str.); ^1H NMR (400 MHz, DMSO-d_6): δ 3.67 (t, 4H, CH_2 morpholine), 3.66 (t, 4H, CH_2 morpholine), 3.83 (s, 3H, -OCH_3), 3.85 (s, 3H, -OCH_3), 6.04 (s, 1H, Ar-H), 8.83 (s, 1H, CH=N), 7.14-8.02 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.7 (OCH_3), 54.4 (OCH_3), 160.6 (C=N, imine), 161.3 (C), 151.4 (C), 161.5 (C), 161.7 (C), 163.4 (C), 48.6 (CH_2), 48.6 (CH_2), 66.4 (CH_2), 66.4 (CH2), 151.5 (C), 100.8 (C), 111.7 (C), 138.4 (CH), 116.4 (CH), 148.7 (CH); MS: *m/z* 415.4 (M^+ +1).

***(E)*-N-((2,4-Dimethoxy-7-morpholino pyrido[2,3-*d*] pyrimidin-6-yl) methylene)-5-methoxypyridin-2-amine, 7e**: Orange solid; m.p.: 176°C. Yield73%. Anal. Calcd for $C_{20}H_{22}N_6O_4$: C, 58.53; H, 5.40; N, 20.48%. Found C, 58.57; H, 5.37; N, 20.45%. IR (KBr): 3075 (C-H str., aromatic), 1478 (C=C str., aromatic), 1557 (C=N str.), 1626 (C=N str., imine), 1189 (OCH_3 str.), 1132 (C-O-C str., morpholine ring), 1377 cm^{-1} (C-N str., morpholine ring);^1H NMR (400 MHz, DMSO-d_6): δ 3.68 (t, 4H, CH_2 morpholine), 3.67 (t, 4H, CH_2 morpholine), 3.87 (s, 3H, -OCH_3), 3.86 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 6.05 (s, 1H, Ar-H), 8.85 (s, 1H, CH=N), 7.14-8.10 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.6 (OCH_3), 54.4 (OCH_3), 55.9 (OCH_3), 161.9 (C=N, imine), 161.2 (C), 153.9 (C), 161.4 (C), 161.5 (C), 163.4 (C), 134.9 (C), 48.6 (CH_2), 48.6 (CH_2), 66.8 (CH_2), 66.8 (CH_2), 131.5 (CH), 100.9 (C), 111.7 (C), 138.5 (CH), 116.7 (CH), 121.5 (CH); MS: *m/z* 411.5 (M^+ +1).

General procedure for the preparation of 2,3-disubstituted-4-thiazolidinone, 8a-e

A mixture of substituted Substituted Schiffbases **(7)** (3.56 g, 1 mol), DMF (50 mL), Pinch of $Zncl_2$ and thioglycolic acid (1.84 g, 2 mol) was refluxed for 10 - 11 hours. Excess solvent was distilled off under reduced pressure. Progress of the reaction was monitored by TLC using ethyl acetate: hexane (4:6). After the completion of the reaction it was cooled and

the product was filtered, washed with dilute sodium bicarbonate solution to remove unreacted acid and dried over anhydrous Na_2SO_4 to get substituted 4-Thiazolidinones derivatives.

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(pyridin-2-yl) thiazolidin-4-one, 8a: Reddish solid; m.p.162°C. Yield 70%. Anal. Calcd for $C_{21}H_{22}N_6O_4S$: C, 55.49; H, 4.88; N, 18.49%. Found C, 55.46; H,4.85; N, 18.46%. IR (KBr): 3054 (C-H str., aromatic), 1466 (C=C str., aromatic), 1542 (C=N str.), 1173 (OCH_3 str.), 1144 (C-O-C str., morpholine ring), 1388 (C-N str., morpholine ring) 1675 (C=O str., thiazolidine), 680 cm^{-1} (C-S-C linkage, thiazolidine ring); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (t, 4H, CH_2, morpholine), 3.44 (t, 4H, CH_2, morpholine), 3.58 (s, 3H, -OCH_3), 3.61 (s, 3H, -OCH_3), 6.30 (s, 1H, Ar-H), 3.92 (concealed doublet, 1H, CHx), 4.68 (concealed doublet, 1H, CHy), 5.75 (s, 1H, CH), 7.42-7.89 (m, 4H, Ar -H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.6 (OCH_3), 54.8 (OCH_3), 33.6 (CH_2, thiazolidine), 127.5 (CH), 124.7 (CH), 134.2 (CH), 146.3 (CH), 68.5 (CH), 66.6 (CH_2), 66.6 (CH_2), 49.3 (CH_2), 49.3 (CH_2), 163.3 (C), 117.6 (C), 159.6 (C), 100.7 (C), 162.5 (C), 161.4 (C), 144.3 (C), 138.8 (CH), 170.9 (C=O, thiazolidine); MS: *m/z* 455.4 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(pyridin-3-yl) thiazolidin-4-one, 8b: Reddish solid; m.p.167°C. Yield 68%. Anal. Calcd for $C_{21}H_{22}N_6O_4S$: C, 55.49; H, 4.88; N, 18.49%. Found C, 55.45; H,4.86; N, 18.45%. IR (KBr): 3061 (C-H str., aromatic), 1467 (C=C str., aromatic), 1552 (C=N str.), 1176 (OCH_3 str.), 1145 (C-O-C str., morpholine ring), 1381 (C-N str., morpholine ring) 1675 (C=O str., thiazolidine), 683 cm^{-1} (C-S-C linkage, thiazolidine ring);^{1}H NMR (400 MHz, DMSO-d_6): δ 3.45 (t, 4H, CH_2, morpholine), 3.47 (t, 4H, CH_2, morpholine), 3.61 (s, 3H, -OCH_3), 3.65 (s, 3H, -OCH_3), 5.76 (s, 1H, Ar-H), 3.92 (concealed doublet, 1H, CHx), 4.68 (concealed doublet, 1H, CHy), 5.76 (s, 1H, CH), 7.42-7.89 (m, 4H, Ar -H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.4 (OCH_3), 54.8 (OCH_3), 33.7 (CH_2, thiazolidine), 127.5 (CH), 124.7 (CH), 134.2 (CH), 146.3 (CH), 68.5 (CH), 66.5 (CH_2), 66.65 (CH_2), 49.4 (CH_2), 49.4 (CH_2), 163.4 (C), 117.6 (C), 159.6 (C), 100.8 (C), 162.7 (C), 161.6 (C), 144.4 (C), 138.7 (CH), 170.9 (C=O, thiazolidine); MS: *m/z* 455.4 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(pyridin-4-yl) thiazolidin-4-one, 8c: Orange solid; m.p.160°C. Yield 75%. Anal. Calcd for $C_{21}H_{22}N_6O_4S$: C, 55.49; H, 4.88; N, 18.49%. Found C, 55.47; H,4.84; N, 18.44%. IR (KBr): 3064 (C-H str., aromatic), 1468 (C=C str., aromatic), 1459 (-C=C-C- str., aromatic), 1554 (C=N str.), 1169 (OCH_3 str.), 1146 (C-O-C str., morpholine ring), 1389 (C-N str., morpholine ring) 1675 (C=O str., thiazolidine), 685 cm^{-1} (C-S-C linkage, thiazolidine ring); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.42 (t, 4H, CH_2, morpholine), 3.44 (t, 4H, CH_2, morpholine), 3.60 (s, 3H, -OCH_3), 3.63 (s, 3H, -OCH_3), 6.32 (s, 1H, Ar-H), 3.92 (concealed doublet, 1H, CHx), 4.68 (concealed doublet, 1H, CHy), 5.75 (s, 1H, CH), 7.13 (d, J=7.42 Hz, 2H, Ar-H), 7.23 (d, J=7.52 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.5 (OCH_3), 54.7 (OCH_3), 33.6 (CH_2, thiazolidine), 127.7 (CH), 124.6 (CH), 134.3 (CH), 146.5 (CH), 68.5 (CH), 66.4 (CH_2), 66.4 (CH_2), 49.6 (CH_2), 49.6 (CH_2), 163.3 (C), 117.6 (C), 159.6 (C), 100.7 (C), 162.8 (C), 161.7 (C), 144.5 (C), 138.8 (CH), 170.9 (C=O, thiazolidine); MS: *m/z* 455.4 (M^+ +1).

3-(5-Chloropyridin-2-yl)-2-(2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl) thiazolidin-4-one, 8d: Light-yellow solid; m.p.157°C. Yield 66%. Anal. Calcd for $C_{21}H_{21}ClN_6O_4S$: C, 51.59; H, 4.33; N, 17.19%. Found C, 51.55; H,4.35; N, 17.16%. IR (KBr): 3066 (C-H str., aromatic), 1458 (C=C str., aromatic), 1553 (C=N str.), 1177 (OCH3 str.), 1149 (C-O-C str., morpholine ring), 1388 (C-N str., morpholine ring) 1679 (C=O str., thiazolidine), 678 (C-S-C linkage, thiazolidine ring) 780 cm^{-1} (C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.40 (t, 4H, CH_2, morpholine), 3.42 (t, 4H, CH_2, morpholine), 3.57 (s, 3H, -OCH_3), 3.62 (s, 3H, -OCH_3), 6.31 (s, 1H, Ar-H), 3.92 (concealed doublet, 1H, CHx), 4.68 (concealed doublet, 1H, CHy), 5.78 (s, 1H, CH), 7.42-7.69 (m, 3H, Ar -H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.5 (OCH_3), 54.7 (OCH_3), 33.6 (CH_2, thiazolidine), 127.7 (CH), 124.6 (CH), 134.3 (CH), 146.5 (CH), 68.5 (CH), 66.4 (CH_2), 66.4 (CH_2), 49.6 (CH_2), 49.6 (CH_2), 163.3 (C), 117.6 (C), 159.6 (C), 100.7 (C), 162.8 (C), 161.7 (C), 144.5 (C), 138.8 (CH), 170.9 (C=O, thiazolidine); MS: *m/z* 489.5 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(3-methoxypyridin-2-yl) thiazolidin-4-one, 8e: Light-yellow solid; m.p.168°C. Yield 73%. Anal. Calcd for $C_{22}H_{24}N_6O_5S$: C, 54.53; H, 4.99; N, 17.34%. Found C, 54.52; H,4.97; N, 17.37%. IR (KBr): 3065 (C-H str., aromatic), 1471 (C=C str., aromatic), 1468 (-C=C-C- str., aromatic), 1549 (C=N str.), 1176 (OCH_3 str.), 1146 (C-O-C str.,

morpholine ring), 1384 (C-N str., morpholine ring) 1679 (C=O str., thiazolidine), 684 cm^{-1} (C-S-C linkage, thiazolidine ring);^{1}H NMR (400 MHz, DMSO-d_6): δ 3.43 (t, 4H, CH_2, morpholine), 3.45 (t, 4H, CH_2, morpholine), 3.60 (s, 3H, -OCH_3), 3.63 (s, 3H, -OCH_3), 3.65 (s, 3H, -OCH_3), 6.34 (s, 1H, Ar-H), 3.92 (concealed doublet, 1H, CHx), 4.68 (concealed doublet, 1H, CHy), 5.77 (s, 1H, CH), 7.42-7.79 (m, 3H, Ar -H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.3 (OCH_3,pyridine), 54.4 (OCH_3), 54.7 (OCH_3), 32.8 (CH_2, thiazolidine), 127.2 (CH), 121.7 (CH), 116.2 (CH), 134.2 (CH), 66.8 (CH_2), 66.8 (CH_2), 49.3 (CH_2), 49.3 (CH_2), 162.5 (C), 117.6 (C), 159.5 (C), 100.7 (C), 163.4 (C), 161.3 (C), 135.4 (C), 151.6 (C), 67.8 (CH), 170.8 (C=O, thiazolidine); MS: *m/z* 485.4 (M^+ +1).

General procedure for the preparation of 2,3-disubstituted-5-methyl-4-thiazolidinone, 9a-e

A mixture of substituted Schiff bases **(7)** (3.56 g, 1 mol), DMF (50 mL), Pinch of $Zncl_2$ and thiolactic acid (1.84g, 2 mol) was refluxed for 10 - 11 hours. Excess solvent was distilled off under reduced pressure. Progress of the reaction was monitored by TLC using ethyl acetate: hexane (4:6). After the completion of the reaction it was cooled and the product was filtered, washed with dilute sodium bicarbonate solution to remove unreacted acid and dried over anhydrous Na_2SO_4 to get substituted 4-Thiazolidinones derivatives.

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-5-methyl-3-(pyridin-2-yl) thiazolidin-4-one, 9a: Light-orange solid; m.p.172°C. Yield 74%. Anal. Calcd for $C_{22}H_{24}N_6O_4S$: C, 56.40; H, 5.16; N, 17.94%. Found C, 56.44; H, 5.12; N,17.97%. IR (KBr): 3079 (C-H str., aromatic), 1566 (C=C str., aromatic), 1679 (C=O str., thiazolidine), 1356 (C-N str.), 1548 (C=N str.), 691 (C-S-C linkage, thiazolidine ring),1138 (C-O-C linkage, morpholine), 1586 (C-N str., morpholine),1166 (-OCH_3 str.),1365 cm^{-1} (CH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.38 (t, 4H, CH_2, morpholine), 3.46 (t, 4H, CH_2, morpholine), 3.69 (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 6.98 (s, 1H, Ar-H), 3.62 (s, 3H, CH_3), 4.2 (q, 1H, J = 7.3 Hz, -CH-CH_3), 5.83 (s, 1H, CH-Ar), 7.42-7.60 (m, 4H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3 (OCH_3), 54.6 (OCH_3), 20.5 (CH_3), 66.5 (CH_2), 66.5 (CH_2), 49.2 (CH_2), 49.2 (CH_2), 65.4 (CH, thiazolidine), 42.6 (CH, thiazolidine), 146.8 (CH), 117.5 (CH), 124.3 (CH), 134.3 (CH), 127.6 (CH), 100.9 (CH), 138.6 (CH), 162.7 (C), 144.3 (C), 161.2 (C), 163.2 (C), 159.6 (C), 173.9 (C=O, thiazolidine); MS: *m/z* 469.5 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-5-methyl-3-(pyridin-3-yl) thiazolidin-4-one, 9b: Ligh- orange solid; m.p.164°C. Yield 69%. Anal. Calcd for $C_{22}H_{24}N_6O_4S$: C, 56.40; H, 5.16; N, 17.94%. Found C, 56.45; H, 5.13; N,17.98%. IR (KBr): 3018 (C-H str., aromatic), 1532 (C=C str., aromatic), 1689 (C=O str., thiazolidine), 1364 (C-N str.), 1559 (C=N str.), 693 (C-S-C linkage, thiazolidine ring),1156 (C-O-C linkage, morpholine), 1575 (C-N str., morpholine), 1173 (-OCH_3 str.),1357 cm^{-1} (CH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.39 (t, 4H, CH_2, morpholine), 3.48 (t, 4H, CH_2, morpholine), 3.67 (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 6.96 (s, 1H, Ar-H), 3.63 (s, 3H, CH3), 4.2 (q, 1H, J = 7.3 Hz, -CH-CH3), 5.86 (s, 1H, CH-Ar), 7.42-7.61 (m, 4H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.4 (OCH_3), 54.7 (OCH_3), 20.6 (CH_3), 66.6 (CH_2), 66.6 (CH_2), 49.4 (CH_2), 49.4 (CH_2), 65.5 (CH, thiazolidine), 42.5 (CH, thiazolidine), 152.5 (CH), 143.6 (CH), 141.6 (CH), 134.4 (CH), 123.7 (CH), 162.8 (C), 117.6 (C), 100.7 (C), 161.3 (C), 163.2 (C), 124.2 (C), 159.4 (C), 173.7 (C=O, thiazolidine); MS: *m/z* 469.7 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-5-methyl-3-(pyridin-4-yl) thiazolidin-4-one, 9c: Light-orange solid; m.p.167°C. Yield 67%. Anal. Calcd for $C_{22}H_{24}N_6O_4S$: C, 56.40; H, 5.16; N, 17.94%. Found C, 56.43; H, 5.19; N,17.91%. IR (KBr): 3055 (C-H str., aromatic), 1553 (C=C str., aromatic), 1692 (C=O str., thiazolidine), 1363 (C-N str.), 1560 (C=N str.), 685 (C-S-C linkage, thiazolidine ring),1147 (C-O-C linkage, morpholine), 1585 (C-N str., morpholine), 1183 (-OCH_3 str.), 1369 cm^{-1} (CH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.36 (t, 4H, CH_2, morpholine), 3.48 (t, 4H, CH_2, morpholine), 3.67 (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 6.98 (s, 1H, Ar-H), 3.64 (s, 3H, CH_3), 4.0 (q, 1H, J = 7.1 Hz, -CH-CH_3), 5.8 4 (s, 1H, CH-Ar), 7.17 (d, J=7.4Hz, 2H, Ar-H), 7.25 (d, J=7.42 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3 (OCH_3), 54.6 (OCH_3), 20.5 (CH_3), 66.5 (CH_2), 66.5 (CH_2), 49.2 (CH_2), 49.2 (CH_2), 65.4 (CH, thiazolidine), 42.6 (CH, thiazolidine), 146.8 (CH), 117.5 (CH), 124.3 (CH), 134.3 (CH), 127.6 (CH), 100.9 (CH), 138.6 (CH), 162.7 (C), 144.3 (C), 161.2 (C), 163.2 (C), 159.6 (C), 173.9 (C=O, thiazolidine); MS: *m/z* 469.6 (M^+ +1).

3-(5-Chloropyridin-2-yl)-2-(2,4-dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-5-methylthiazolidin-4-one, 9d: Light-orange solid; m.p.152°C. Yield 71%. Anal. Calcd for $C_{22}H_{23}ClN_6O_4S$: C, 52.53; H, 4.61; N, 16.71%. Found C, 52.57; H, 4.65; N,16.75%. IR (KBr): 3052 (C-H str., aromatic), 1566 (C=C str., aromatic), 1687 (C=O str., thiazolidine), 1336 (C-N str.),1539 (C=N str.), 688 (C-S-C linkage, thiazolidine ring), 1128 (C-O-C linkage, morpholine), 1573 (C-N str., morpholine), 1167 (-OCH_3 str.), 1358 (CH_3 str.), 667 cm^{-1} (C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.40 (t, 4H, CH_2, morpholine), 3.49 (t, 4H, CH_2, morpholine), 3.69 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3), 6.97 (s, 1H, Ar-H), 3.64 (s, 3H, CH_3), 4.0 (q, 1H, J = 7.1 Hz, -CH-CH_3), 5.85 (s, 1H, CH-Ar), 7.42-7.60 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3 (OCH_3), 54.6 (OCH_3), 20.5 (CH_3), 66.5 (CH_2), 66.5 (CH_2), 49.2 (CH_2), 49.2 (CH_2), 65.4 (CH, thiazolidine), 42.6 (CH, thiazolidine), 146.8 (CH), 117.5 (CH), 124.3 (CH), 134.3 (CH), 127.6 (CH), 100.9 (CH), 138.6 (CH), 162.7 (C), 144.3 (C), 161.2 (C), 163.2 (C), 159.6 (C), 173.9 (C=O, thiazolidine); MS: *m/z* 504.4 (M^+ +1).

2-(2,4-Dimethoxy-7-morpholinopyrido[2,3-*d*]pyrimidin-6-yl)-3-(3-methoxypyridin-2-yl) -5-methylthiazolidin-4-one, 9e: Light-orange solid; m.p.171°C. Yield 68%. Anal. Calcd for $C_{22}H_{26}N_6O_5S$: C, 55.41; H, 5.26; N, 16.86%. Found C, 55.45; H, 5.29; N,16.83%. IR (KBr):3068 (C-H str., aromatic), 1565 (C=C str., aromatic), 1689 (C=O str., thiazolidine), 1338 (C-N str.), 1548 (C=N str.), 698 (C-S-C linkage, thiazolidine ring),1127 (C-O-C linkage, morpholine), 1575 (C-N str., morpholine), 1188 (-OCH_3 str.), 1359 cm^{-1} (CH_3 str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.37 (t, 4H, CH_2, morpholine), 3.48 (t, 4H, CH_2, morpholine), 3.66 (s, 3H, -OCH_3), 3.78 (s, 3H, -OCH_3), 3.69 (s, 3H, -OCH_3), 6.98 (s, 1H, Ar-H), 3.64 (s, 3H, CH_3), 4.1 (q, 1H, J = 7.1 Hz, -CH-CH_3), 5.8 5(s, 1H, CH-Ar), 7.42-7.63 (m, 3H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3 (OCH_3), 54.6 (OCH_3), 20.5 (CH_3), 66.5 (CH_2), 66.5 (CH_2), 49.2 (CH_2), 49.2 (CH_2), 65.4 (CH, thiazolidine), 42.6 (CH, thiazolidine), 146.8 (CH), 117.5 (CH), 124.3 (CH), 134.3 (CH), 127.6 (CH), 100.9 (CH), 138.6 (CH), 162.7 (C), 144.3 (C), 161.2 (C), 163.2 (C), 159.6 (C), 173.9 (C=O, thiazolidine); MS: *m/z* 499.6 (M^+ +1).

Pharmacological studies

Antibacterial and antifungal activity

In vitro antibacterial and antifungal activity of newly synthesized compounds **7a-e**, **8a-e** and **9a-e** was carried out by micro broth dilution method[26]. A panel of selected pathogens Gram positive (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442) and Gram negative (*Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 441) bacterial species used for antibacterial activity whereas for antifungal activity, a panel of selected fungal pathogens (Candida albicans MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus calavatus* MTCC 1323) species were used. 2% DMSO solution was used as diluent to get desired concentration of drugs to test upon standard bacterial and fungal strains. The zone of inhibition produced by each compound was measured in µg/mL. The minimum inhibitory concentration (MIC) was determined and recorded at the lowest concentration inhibiting growth of the organism. Ampicillin, Chloramphenicol and Ciprofloxacin were used as standard antibiotic drugs for antibacterial activity while Greseofulvin and Nystatin were used as standard drug for antifungal activity. The results are summarised in Table 1.

Antitubercular activity

In vitro antitubercular activity of all the newly synthesized compounds was determined by using Lowenstein-Jensen medium (conventional method) against *Mycobacterium tuberculosis* H37Rv strain[27]. The observed results are presented in Table 1 in the form of inhibition (%), relative to that of standard antitubercular drugs Isoniazid and Rifampicin. Compounds demonstrating more than 80% inhibition in the primary screening were retested at lower concentration (MIC) in a Lowenstein-Jensen medium and evaluated for their MIC values. Five compounds exhibiting more than 80% inhibition were again screened to get their MIC values (Table 2).

Evaluation of Antibacterial activity

Upon reviewing *in vitro* antibacterial activity data (Table 1) it has been observed that in Gram positive bacterial strains, compounds **7c**, **8c**, **9c**, **7d**, and **7e** displayed an outstanding inhibitory effect *i.e.* MIC = 50 µg/mL and 62.5 µg/mL respectively against *Staphylococcus aureus* compared to Ampicillin (MIC = 250 µg/mL) and admirable inhibitory effect to Chloramphenicol and Ciprofloxacin (MIC = 50 µg/mL). Compounds **7a**, **7b**, **8a**, and **9d** (MIC = 100 µg/mL), **8d**, and **9e** (MIC = 125 µg/mL), **9b** (MIC = 200 µg/mL) were found to possesses significant activity compared to Ampicillin (MIC = 250 µg/mL) while compounds **8b** (MIC = 250 µg/mL) showed the same potency as

Table 1 — *In vitro* antitubercular activity data of the synthesised compounds **7a-e**, **8a-e** and **9a-e** exhibiting against *M. tuberculosis* H37Rv (MICs, µg/mL)

Compd	Antimicrobial activity (MIC) µg/mL							Antitubercular activity
	Antibacterial activity				Antifungal activity			
	Gram Positive Bacteria		Gram Negative Bacteria		Fungus			Inhibition at 250 µg/mL (%)
	S. aureus	*S. pyogenes*	*E. coli*	*P. aerug.*	*C. albican*	*A. niger*	*A. clavatus*	*M. tuberculosis H37Rv*
7a	100	250	250	200	500	200	250	82
7b	100	200	500	100	250	100	500	64
7c	50	200	100	250	500	250	100	68
7d	62.5	250	200	100	500	1000	>1000	87
7e	62.5	200	62.5	100	500	100	200	88
8a	100	500	200	200	1000	1000	500	53
8b	250	100	62.5	100	500	200	100	67
8c	50	62.5	100	125	100	250	100	68
8d	125	100	100	150	>1000	>1000	>1000	85
8e	62.5	100	250	250	1000	100	500	91
9a	150	250	100	100	500	1000	1000	66
9b	200	100	250	62.5	100	100	500	63
9c	50	250	125	200	250	500	100	87
9d	100	62.5	100	250	500	>1000	>1000	89
9e	125	200	100	62.5	500	500	500	69
A	250	100	100	100	–	–	–	–
B	50	50	50	50	–	–	–	–
C	50	50	25	25	–	–	–	–
D	–	–	–	–	500	100	100	–
E	–	–	–	–	100	100	100	–
F	–	–	–	–	–	–	–	99
G	–	–	–	–	–	–	–	98

A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Greseofulvin, E: Nystatin, F: Isoniazid and G: Rifampicin.

Table 2 — *In vitro* antitubercular activity data of the synthesised compounds exhibiting greater inhibition against *M. tuberculosis* H37Rv (MICs, µg/mL)

Compd	% Inhibition	MIC (µg/mL)
7a	82	50
7d	87	100
7c	88	62.5
8d	85	50
8e	91	50
9c	87	62.5
9d	89	62.5
A	99	0.20
B	98	40

compared to Ampicillin (MIC = 250 µg/mL) against *Staphylococcus aureus*. Against *Streptococcus pyogenes*, compound **8c** and **9d** (MIC = 62.5 µg/mL) exerted the highest activity compared to Ampicillin (MIC = 100 µg/mL) and lowest to Chloramphenicol and Ciprofloxacin (MIC = 50 µg/mL) while compounds **8b**, **8d**, **8e**, and **9b** (MIC = 100 µg/mL) showed equipotent potency compared to Ampicillin (MIC = 100 µg/mL) against the same organism *Streptococcus pyogenes*.

In the case of inhibiting Gram-negative bacteria, compound **7e** and **8b** (MIC = 62.5 µg/mL) exhibited an outstanding inhibitory effect whereas compounds **7c**, **8c**, **8d**, **9a**, **9d** and **9e** (MIC = 100 µg/mL) demonstrated the same potency compared to Ampicillin (MIC = 100 µg/mL) against *Escherichia coli*. Against *Pseudomonas aeruginosa*, compound **9b** and **9e** (MIC = 62.5 µg/mL) exhibited excellent activity while compounds **7b**, **7d**, **7e**, **8b** and **9a** displayed equipotent activity as compared to Ampicillin (MIC = 100 µg/mL). The remaining compounds showed moderate to good activity that inhibit the growth of bacterial pathogens and were found less effective than the employed standard drugs.

Evaluation of antifungal activity

The antifungal screening data in Table 1 revealed that compounds **7b** and **9c** (MIC = 250 µg/mL) displayed an excellent inhibitory effect compared to Greseofulvin (MIC = 500 µg/mL) against *Candida albicans* whereas compounds **7a**, **7c**, **7d**, **7e**, **8b**, **9a**, **9d** and **9e** (MIC = 500 µg/mL) exerted comparable activity as compared to Greseofulvin (MIC = 500 µg/mL) against same fungal

pathogen. Against *Aspergillus niger*, compounds **7a** and **8c** (MIC = 250 µg/mL) and **7c** and **8b** (MIC = 200 µg/mL) were found to possess significant activity to Greseofulvin and Nystatin (MIC = 100 µg/mL). Against *Aspergillus clavatus*, compounds **7a** (MIC = 250 µg/mL) showed resonable activity compared to Greseofulvin and Nystatin (MIC = 100 µg/mL). The remaining compounds showed moderate to good activity against all fungal strain.

Evaluation of antitubercular activity

The encouraging results of the antimicrobial screening prompted us to screen the title compounds for their *in vitro* antitubercular activity. Compounds demonstrating more than 80% inhibition in the primary screening were retested at lower concentration (MIC) in a Lowenstein–Jensen medium and evaluated for their MIC values. The result presented in Table 1 reveals that five compounds exhibiting more than 80% inhibition were again screened to get their MIC values (Table 2). Among the compounds screened for antitubercular activity, compounds **7a**, **8d** and **8e** (MIC = 50 µg/mL), **7d** (MIC = 100 µg/mL), **7c** (MIC = 62.5 µg/mL), **9c** (MIC = 62.5 µg/mL) and **9d** (MIC = 62.5 µg/mL) were found to possess the greatest potency against *Mycobacterium tuberculosis* with **82**, **85**, **91**, **87**, **88**, **87** and **89**% inhibition respectively (Table 2). Other derivatives showed moderate to poor antitubercular activity. Compounds **7a**, **7d**, **7e**, **8d**, **8e**, **9c** and **9d** showed comparable activity to the standard drug. Alas, other derivatives showed moderate to poor antitubercular activity.

Conclusion

Synthesized and structurally characterizedusing spectroscopic techniques. The imine derivatives containing pyrido[2,3-*d*]pyrimidine moiety have been evaluated *in vitro* for their antimicrobial activities against bacterial strains (*Staphylococcus aureus*, *Streptococcus pyogene*s, *Pseudomonas aeruginosa*, and *Escherichia coli*) microorganisms. The results showed that majority of the novel imine derivatives have good antibacterial activity among them **7e** exhibite the most significant activity compared to the parent drug.

The study enriched the knowledge of novel heterocyclic compounds as possible antibacterial agents. All the synthesis compound possessing electron donating or withdrawing atom/group such as methyl, methoxy, chloro, *etc*. have been identified as the most potent antibacterial and antifungal agents and identified as most proficient member of the series. Compounds **7a**, **7d**, **7c**, **8d**, **8c**, **9c**, and **9d** showed excellent antitubercular activity.

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