SYNTHESIS AND ANTIMICROBIAL ACTIVITIY OF NOVEL BARBITURIC ACID AND THIOHYDANTOIN DERIVATIVES OF IMIDAZO [2, 1- B][1, 3, 4] THIADIAZOLES.

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ABSTRACT:

A series 5-[6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl-methylene]-pyrimidine-2,4,6-triones (4a-f) and 5-[6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl]methylene-2-thioxoimidazolidin-4-one (5a-f) were synthesized from 5-formyl derivatives of 2-(4-methoxybenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole (3a-f) by the knoevenagel condensation with barbituric acid & thiohydantoin. The required 5-formyl derivatives were prepared by the Vilsmeier Haack reaction of the corresponding 2,6-disubstituted imidazo[2,1-b][1,3,4]thiadiazoles (2a-f). The structures of all the newly synthesized compounds were established by analytical and spectral data. The synthesized compounds were screened for their antibacterial and antifungal activities.

Key Words: Imidazo[2,1-b][1,3,4]thiadiazole; VilsmeierHaack reaction; Imidazo[2,1- b][1,3,4] thiadiazole-5-carbaldehydes; Knoevenagel condensation; Pyrimidine-2,4,6-trione derivatives; Thioxoimidazolidinones; Antibacterial activity, Antifungal activity.

INTRODUCTION

Imidazo[2,1-b][1,3,4]thiadiazole derivatives[1-4] occupy a prominent place in medicinal chemistry because of their significant properties as therapeutics. In the field of pharmaceutical chemistry the derivatives of barbituric acid [5, 6] have occupied special place. Their biological activities range from classical applications as hypnotics to sedatives and anesthic drugs. More recently there are reports of its applications as anticancer and antisteoporosis agents. In the present investigation we have synthesized novel imidazothiadiazoles (2a-f) and subjected them to Vilsmeier Haack reaction resulting in formation of 5-formyl derivatives.

Knoevenagel condensation of 6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4] thiadiazole-5-carbaldehydes(3a-f)with barbituric acid leads to the formation of novel Pyrimidine-2,4,6-trione derivatives, similarly on condensation with thiohydantoin [7], the thioxoimidazolidinone derivatives were obtained, thereby creating significant interest in their pharmacological properties [8-12].

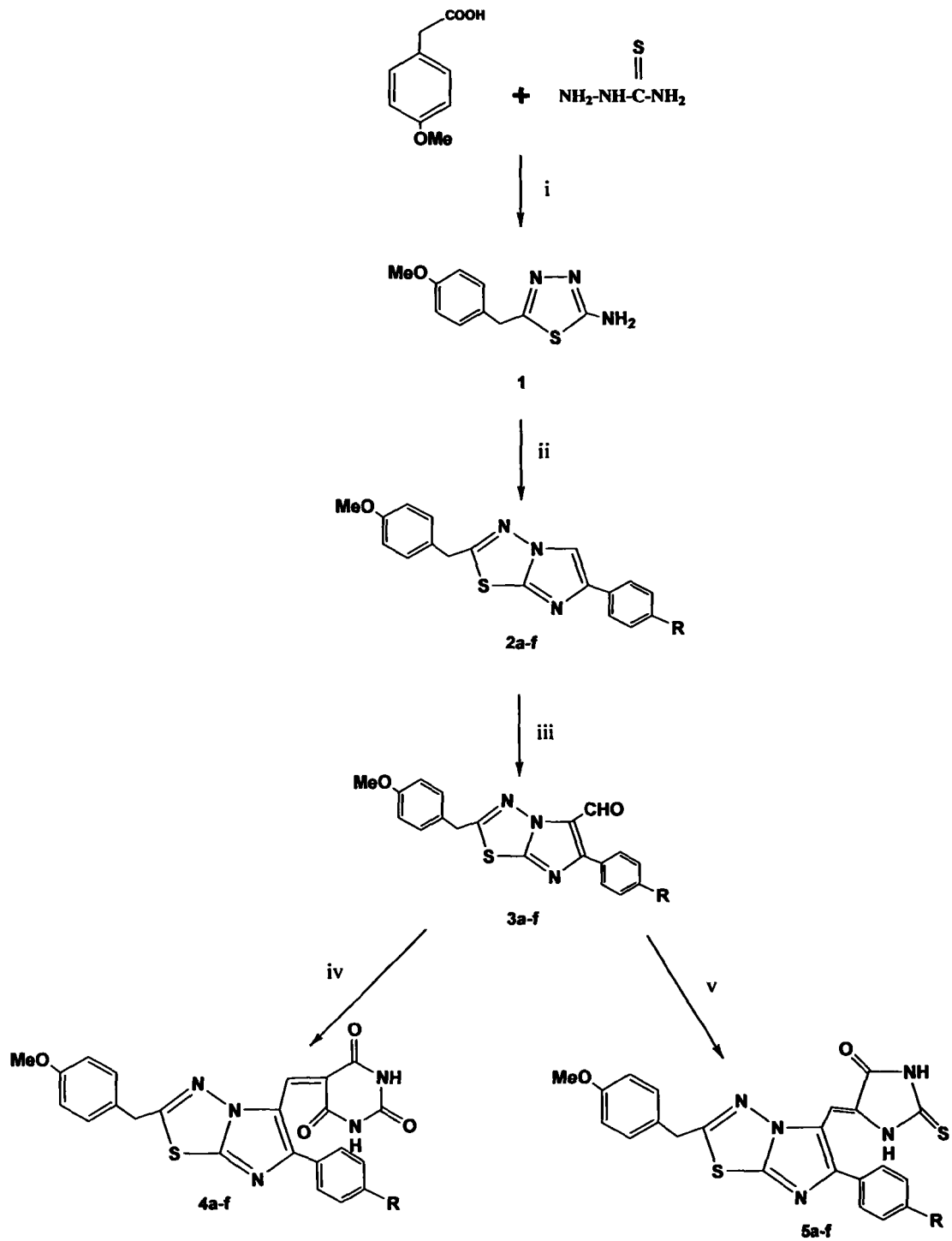
RESULTS AND DISCUSSION

During the present investigation required imidazo[2,1-*b*][1,3,4]thiadiazoles were prepared by the reaction of 2-amino-1,3,4-thiadiazole (**1**) with appropriately substituted α-haloketones (phenacylbromides) in dry ethanol as hydrobromides, which on neutralization with aqueous sodium carbonate gave corresponding free bases (**2a-f**) in good

yields. The absence of ν_{N-H} band in IR spectra of the resulted compounds confirms the formation of product, which exhibits imidazole (C_5-H) proton around δ 7.95 in ^{1}H NMR spectra. The ^{13}C NMR spectrum of compounds is in total agreement with the structures.

Imidazo[2,1-*b*][1,3,4]thiadiazoles (**2a-f**) were further subjected to Vilsmeir Haack reaction, which resulted in the formation of expected imidazo[2,1-*b*][1,3,4] thiadiazole-5-carbaldehydes (**3a-f**) and were confirmed by their spectral and analytical data. The IR spectra of these compounds displayed the aldehydic carbonyl around 1680cm^{-1} and ν_{C-H} around 2850cm^{-1}. The structures were further confirmed by the presence of a signal around δ 10.00 for aldehydic proton and absence of C_5-H of imidazole in the ^{1}H NMR spectra. Further ^{13}C NMR spectra exhibited carbonyl carbon around δ 181.2 and rest of the carbons resonated in the expected region.

The intermediates were exploited by Knoevenagel condensation with Barbituric acid & Thiohydantoin. The reaction underwent smoothly with excellent yields. The formation of compound can be observed in the reaction mixture itself as they come out as intense yellow solid from the clear solution within few minutes. The formation of 5-[6-aryl-2-(4-methoxybenzyl)-imidazo[2,1-b][1,3,4]thiadiazol-5-yl-methylene]-pyrimidine-2,4,6 triones (**4a-f**) were confirmed by their IR spectra, which displayed the $\nu_{C=O}$ bands around 1735 and 1698cm^{-1} & ν_{N-H} bands around 3186, 3031. Further, they were confirmed by ^{1}H NMR spectra, where aldehydic proton disappeared and the vinylic proton resonated in the region δ 8.40-8.50. Appearance of two N-H protons in the region of 11.03-11.08 & 11.27-11.58 further confirmed the formation of these compounds. Similarly the formation of 5-[6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]methylene-2-thioxoimidazolidin-4-one (**5a-f**) were confirmed by their IR spectra, which displayed the $\nu_{C=O}$ bands around 1735 and 1698cm^{-1}. Further, they were confirmed by ^{1}H NMR spectra, where aldehydic proton disappeared and the vinylic proton resonated in the region δ 8.50-8.55. Appearance of two N-H protons in the region of 11.03-11.20 & 11.27-11.58 further confirmed the formation of these compounds. All the derivatives (**4a-f & 5a-f**) are having high melting points compared to its starting materials due to formation of rigid and very stable compound. The structures of all the newly synthesized compounds were established by their spectral and analytical data.



Scheme-1

Reagents & Conditions: i, $POCl_3$ reflux, NH_3.

ii, $ArCOCH_2Br$,dry EtOH reflux.

iii, $DMF/POCl_3$,Na_2CO_3.

iv, Barbituric acid, AcOH, Sodiumacetate, reflux.

v, Thiohydantoin, AcOH, Sodiumacetate, reflux.

a, R= Cl; b, R= Br; c, R= H; d, R= Me; e, R= OMe; f, R= NO_2.

EXPERIMENTAL

4-Methoxyphenylacetic acid, barbituric acid, thiohydantoin and all reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd India. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on Nicolet Impact-410 FT-IR spectrophotometer, using KBr pellet technique. ^{1}H NMR and ^{13}C NMR experiments were performed on a 300 MHz on Bruker AC-300F spectrometer (TMS as internal standard). All the newly synthesized compounds were analyzed for C, H, N and results were found to be within the allowed limit.

Synthesis of 5-(4-methoxybenzyl)-1,3,4-thiadiazol-2-amine (1)

A mixture of 4-methoxyphenylacetic acid (1.67g ; 10 mmol) and Thiosemicarbazide (0.92 g ; 10 mmol) in phosphorous oxychloride (30 mL) was refluxed gently for 45 mins. The reaction mixture was cooled and quenched (highly exothermic) with cold water (90 mL). The resulting solution was refluxed for additional 4 hours and filtered hot. The filtrate was cooled and basified with aqueous potassium hydroxide solution. The solid that separated was filtered, washed with water, dried and recrystallised from ethanol. Colourless flakes (ethanol), Yield 90%, m.p. 195-197°C; IR (KBr) υ 3257, 3105, 2972, 2927 cm^{-1}; ^{1}H NMR(300MHz, $CDCl_3$) δ: 3.81(s, 3H, OCH_3), 4.17(s, 2H, CH_2), 5.1(s, 2H, NH_2), 6.86-7.28(m, 4H, Ar-H); Anal. Calcd. for $C_{10}H_{11}N_3OS$; C, 54.28; H, 5.01; N, 18.99%. Found: C, 54.10 ; H, 4.91; N, 18.50%.

Synthesis of 2-(4-methoxybenzyl)-6-aryl-imidazo[2,1-b][1,3,4]thiadiazole (2a-f)

General Procedure

A mixture of equimolar quantities of 5-(4-methoxybenzyl)-1,3,4-thiadiazol-2-amine (0.01 mol) and appropriate bromoacetyl compound (10 mmol) were refluxed in dry ethanol for 8 hours. The excess of solvent was distilled off and the hydrobromide salt that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution to get free base. It was filtered, washed with water, dried and recrystalised from suitable solvent.The physicochemical and analytical data of various **2a-f** are described below.

6-(4-chlorophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole (2a)

Pale yellow solid (ethanol), Yield 75%, m.p.141-142^{0}C; IR (KBr) υ 1512, 1610, 2912 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.8(s, 3H, OCH_3), 4.2(s, 2H, CH_2), 7.24-7.78(m, 8H, Ar-H), 7.98(s, 1H, C_5-H Imidazole); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.80(OCH_3), 55.71(CH_2), 109.58, 127.47, 129.2, 133.5, 165.1, 159.7 and 146.4; Anal. Calcd. for $C_{18}H_{14}N_3SOCl$; C, 60.76; H, 3.97; N, 11.81%. Found: C, 60.70 ; H, 3.91; N, 11.2%.

6-(4-bromophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole (2b)

Colorless solid (ethanol), Yield 80%, m.p.158-160^{0}C; IR (KBr) υ 1510, 1612, 2912 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.8(s, 3H, OCH_3), 4.23(s, 2H, CH_2), 7.24-7.78(m, 8H, Ar-H), 7.98(s, 1H, C_5-H Imidazole); Anal. Calcd. for $C_{18}H_{14}N_3SOBr$; C, 54.01; H, 3.53;
N,10.50%. Found: C, 54.08 ; H, 3.46; N, 10.2%.

2-(4-methoxybenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole (2c)

Colorless solid (ethanol), Yield 90%, m.p.196^{0}C; IR (KBr) υ 1510, 1612, 2912 cm^{-1};
^{1}H NMR(300MHz, $CDCl_3$) δ: 3.83(s,3H,OCH_3), 4.26(s, 2H, CH_2), 6.90-7.82(m, 9H, Ar-H), 7.97(s, 1H, C_5-H Imidazole); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.85(OCH_3), 55.69(CH_2),109.38, 127.47, 129.6, 132.6, 164.8, 159.9 and 146.2; Anal. Calcd. for $C_{18}H_{15}N_3OS$; C, 67.27; H, 4.70; N, 13.07%. Found: C, 67.20 ; H, 4.46; N, 13.02%

2-(4-methoxybenzyl)-6-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (2d)

Pale Yellow solid (ethanol), Yield 90%, m.p.163^{0}C; IR (KBr) υ 1502, 1613, 2920 cm^{-1};

^{1}H NMR(300MHz, $CDCl_3$) δ: 2.38(s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 4.25(s, 2H, CH_2), 6.90-7.71(m, 8H, Ar-H), 7.93(s, 1H, C_5-H Imidazole); Anal. Calcd. for $C_{19}H_{17}N_3OS$; C, 68.03; H, 5.11; N, 12.53%. Found: C, 67.90 ; H, 5.06; N, 12.42%.

2-(4-methoxybenzyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (2e)

Pale Yellow solid (ethanol), Yield 80%, m.p.210^{0}C; IR (KBr) υ 1502, 1612, 2930 cm^{-1};

^{1}H NMR(300MHz, $CDCl_3$) δ: 3.83(d, 6H, OCH_3), 4.25(s, 2H, CH_2), 6.90-7.75(m, 8H, Ar-H), 7.88(s, 1H, C_5-H Imidazole); ^{13}C NMR(75MHz, $CDCl_3$) δ: 21.02(CH_3), 37.85(OCH_3), 37.86(OCH_3), 55.69(CH_2), 109.38, 127.47, 129.6, 132.6, 164.8, 159.9 and 146.2; Anal. Calcd. for $C_{19}H_{17}N_3O_2S$; C, 64.94; H, 4.88; N, 11.96%.Found: C, 64.90 ; H, 4.80; N, 11.42%.

2-(4-methoxybenzyl)-6-(4-nitrophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole (2f)

Brown solid (ethanol), Yield 75%, m.p.183^{0}C; IR (KBr) υ 1510, 1610, 2930 cm^{-1};

^{1}H NMR(300MHz, $CDCl_3$) δ: 3.87(s, 3H, OCH_3), 4.27(s, 2H, CH_2), 6.90-8.29(m, 8H, Ar-H), 8.11(s, 1H, C_5-H Imidazole); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.82(OCH_3), 55.73(CH_2), 109.38, 127.47, 129.6, 132.5, 164.1, 159.7 and 146.8; Anal. Calcd. for $C_{18}H_{14}N_4O_3S$; C, 59.01; H, 3.85; N, 15.29%. Found: C, 59.08 ; H, 3.76; N, 15.20%.

Synthesis of 2-(4-methoxybenzyl)-6-aryl-imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes (3a-f), General Procedure

Vilsmeier Haack reagent was prepared by adding phosphorylchloride (3mL) in dimethylformamide (20mL) at 0°C with stirring. Then appropriately substituted 2-(4-methoxybenzyl)-6-(4-methylphenyl)imidazo[2,1b][1,3,4]thiadiazole **(2a-f)** (10 mmol) was added to the reagent and stirred at 0°C for 30 minutes. The mixture was further stirred for 2 hours at room temperature and at 60°C for additional 2 hours. The reaction mixture was then poured in sodium carbonate solution and stirred at 90°C for 2 hours. After cooling, the mixture was diluted with water, extracted with chloroform and the collective extract was washed with water, dried over anhydrous sodium sulphate. The residue obtained after removal of chloroform was recrystallized from suitable solvent to get the crystalline solids. Various formyl derivatives obtained are described below.

6-(4-chlorophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5- carbaldehyde (3a)

Colorless flakes (Chloroform), Yield 81%, m.p.122^{0}C; IR (KBr) υ 1683, 2923, 1512, 1611 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.83(s, 3H, OCH_3), 4.27(s, 2H, CH_2), 6.92-7.8(m, 8H, Ar-H),10.06 (s,1H,CHO); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.80(OCH_3), 55.8(CH_2), 109.6, 128.4, 133.6, 165.1, 146.0 & 180.2(CHO); Anal. Calcd. for $C_{19}H_{14}N_3SO_2Cl$; C, 59.45; H, 3.68; N, 10.95%. Found: C, 59.30 ; H, 3.60; N, 10.86%.

6-(4-bromophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (3b)

Colorless solid (Chloroform), Yield 86%, m.p.135^{0}C; IR (KBr) υ 2923, 1512, 1663, 1682 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.83(s , 3H, OCH_3), 4.32(s, 2H, CH_2), 6.9-7.82(m, 8H, Ar-H), 10.02(s, 1H, CHO); ^{13}C NMR(75MHz , $CDCl_3$) δ: 37.80(OCH_3), 56.2(CH_2),109.6, 128.4, 133.6, 169.1, 146.0 & 181.2(CHO); Anal. Calcd. for $C_{19}H_{14}N_3SO_2Br$; C, 53.48; H, 3.29; N, 9.81%. Found: C, 53.30 ; H, 3.10; N, 9.86%.

2-(4-methoxybenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (3c)

Colorless solid (Chloroform), Yield 92%, m.p.120^{0}C; IR (KBr) υ 2923, 1512, 1608, 1673 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.83(s, 3H, OCH_3), 4.41(s, 2H, CH_2), 6.9-7.84(m, 9H, Ar-H), 10.04(s ,1H, CHO); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.80(OCH_3), 56.2(CH_2),109.6, 128.4, 129.5, 133.6, 169.1, 146.0 & 181.2(CHO); Anal. Calcd. for $C_{19}H_{15}N_3O_2S$; C, 65.31; H, 4.33; N, 12.03%. Found: C, 65.30 ; H, 4.20; N, 12.86%.

2-(4-methoxybenzyl)-6-(4-methylphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (3d)

Pale Yellow solid (Chloroform), Yield 90%, m.p.135^{0}C; IR (KBr) υ 2923, 1512, 1683 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 2.23(s, 3H, CH_3), 3.83(s, 3H,OCH_3), 4.41(s ,2H, CH_2),6.9-7.84(m, 8H, Ar-H), 10.02(s,1H,CHO); Anal. Calcd. for $C_{20}H_{17}N_3O_2S$; C, 66.10; H, 4.71; N, 11.56%. Found: C, 66.00 ; H, 4.35; N, 11.31%.

2-(4-methoxybenzyl)-6-(4-methoxyphenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (3e)

Pale Yellow solid (Chloroform), Yield 85%, m.p.147^{0}C; IR (KBr) υ 2923, 1512, 1663, 1680 cm^{-1};^{1}H NMR(300MHz, $CDCl_3$) δ: 3.80(d, 6H, OCH_3), 4.42(s , 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 10.02(s,1H,CHO); ^{13}C NMR(75MHz, $CDCl_3$) δ: 22.01(CH_3), 37.80(OCH_3), 37.81(OCH_3), 56.2(CH_2), 109.6, 128.4, 133.6, 169.1, 146.0 &181.2(CHO); Anal. Calcd. for $C_{20}H_{17}N_3O_3S$; C, 63.31; H, 4.52; N, 11.07%. Found: C, 63.10 ; H, 4.20; N, 11.06%.

2-(4-methoxybenzyl)-6-(4-nitrophenyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehyde (3f)

Brown solid (Chloroform), Yield 91%, m.p.126^{0}C; IR (KBr) υ 2923, 1512, 1608, 1682 cm^{-1};^{1}H NMR (300MHz, $CDCl_3$) δ: 3.83(s, 3H, OCH_3), 4.40(s , 2H, CH_2), 6.9-8.33(m, 8H, Ar-H), 10.18(s, 1H, CHO); ^{13}C NMR(75MHz, $CDCl_3$) δ: 37.80(OCH_3), 56.2(CH_2),109.6, 128.4, 133.6, 169.1, 146.0 & 181.2(CHO);); Anal. Calcd. for $C_{19}H_{14}N_4O_4S$; C, 57.86; H, 3.58; N, 14.2%. Found: C, 57.30 ; H, 3.25; N, 13.86%.

Synthesis of 5-[6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl-methylene]pyrimidine 2,4,6-triones. (4a-f), General Procedure

A mixture of 2-(4-methoxybenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde **(3a-f)** (0.01 mol) and Barbituric acid (1.28 g ; 0.01 mol) in glacial acetic acid (10 mL) with catalytic amount of sodium acetate in glacial acetic acid (5 mL) was refluxed for 2 hours. The reaction mixture was allowed to cool. The red solid that separated was filtered, dried & recrystallised from suitable solvent to get various **4a-f.**

5-{[6-(4-chlorophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl] methylene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4a)

Brown red solid(Chloroform), Yield 90%, m.p.310^{0}C; IR (KBr) υ 3186, 3101, 2823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.82(s, 3H,OCH_3), 4.13(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.5(s, 1H, =CH), 11.19 (s, 1H, NH), 11.57(s,1H, NH); ^{13}C NMR(75MHz, DMSO) δ: 42.7 (CH_2), 56.0(OCH_3), 114.0, 122, 128.4, 129.4, 147.4, 157.2, 166.3; Anal. Calcd. for $C_{23}H_{16}ClN_5O_4S$; C, 55.93; H, 3.27; N, 14.18%. Found: C, 55.40 ; H, 3.15; N, 13.31%.

5-{[6-(4-bromophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl] methylene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4b)

Brown solid (Chloroform), Yield 90%, m.p.296^{0}C; IR (KBr) υ 3186, 3101, 2823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(s, 3H,OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.52(s, 1H, =CH), 11.18 (s, 1H, NH), 11.47(s, 1H, NH); Anal. Calcd. for $C_{23}H_{16}BrN_5O_4S$; C, 51.31; H, 3.00; N, 13.01%. Found: C, 51.20 ; H, 3.05; N, 12.81%.

5-{[2-(4-methoxybenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl] methylene} pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4c)

Brown red solid(Chloroform), Yield 90%, m.p.316^{0}C; IR (KBr) υ 3216, 3101,2 823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(s, 3H,OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 9H, Ar-H), 8.52(s, 1H, =CH), 11.18 (s, 1H, NH), 11.47(s, 1H, NH); Anal. Calcd. for $C_{23}H_{17}N_5O_4S$; C, 60.12; H, 3.73; N, 15.24%. Found: C, 60.20 ; H, 3.25; N, 14.81%.

5-{[6-(4-methylphenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5- yl] methylene} pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4d)

Red solid (Chloroform), Yield 95%, m.p.296^{0}C; IR (KBr) υ 3216, 3101, 2823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 2.35(s, 3H,CH_3), 3.83(s, 3H,OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.45(s, 1H, =CH), 11.06 (s, 1H, NH), 11.27(s, 1H, NH); ^{13}C NMR(75MHz, DMSO) δ: 20.9(CH_3), 42.7 (CH_2), 56.0(OCH_3), 114, 122, 126.9, 129.7, 147.4, 157.2, 163.7, 166.3; Anal. Calcd. for $C_{24}H_{19}N_5O_4S$; C, 60.88; H, 4.04; N, 14.79%. Found: C, 60.70 ; H, 4.25; N, 14.81%.

5-{[6-(4-methoxyphenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4] thiadiazol-5-yl]methylene} pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4e)

Brown red solid(Chloroform), Yield 90%, m.p.310^{0}C; IR (KBr) υ 3216, 3101, 2823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(d, 6H, OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.55(s, 1H, =CH), 11.06 (s,

1H, NH), 11.27(s, 1H, NH); Anal. Calcd. for $C_{24}H_{19}N_5O_5S$; C, 58.89; H, 3.91; N, 14.31%. Found: C, 58.70 ; H, 3.25; N, 14.11%.

5-{[6-(4-nitrophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl] methylene}pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (4f)

Red solid (Chloroform), Yield 96%, m.p.286^{0}C; IR (KBr) υ 3216, 3101, 2823, 1736, 1512, 1608 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(d, 6H, OCH_3), 4.42(s, 2H, CH_2), 6.92-7.84(m,8H, Ar-H), 8.55(s, 1H, =CH), 11.06 (s, 1H, NH), 11.27(s, 1H, NH); ^{13}C NMR(75MHz, DMSO) δ: 42.7 (CH_2), 56.0(OCH_3), 114, 122, 124.1, 127.9, 136, 147.4, 157.2, 163.7, 166.3; Anal. Calcd. for $C_{24}H_{16}N_6O_6S$; C, 54.76; H, 3.20; N, 16.66%. Found: C, 54.70 ; H, 3.25; N, 16.41%.

Synthesis of 5-[6-aryl-2-(4-methoxybenzyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]methylene-2-thioxoimidazolidin-4-one.(5a-f), General Procedure

A mixture of 2-(4-methoxybenzyl)-6-arylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde **(3a-f)** (10 mmol) & Thiohydantoin (1.16 g ; 0.01 mol) in glacial acetic acid (10 mL) with catalytic amount of sodium acetate in glacial acetic acid (5 mL) refluxed for 2 hours. The reaction mixture was allowed to cool. The yellow solid that separated was filtered, dried & recrystallised from suitable solvent to get various **5a-f** described below.

5-{[6-(4-chlorophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5a)

Yellow solid (Chloroform), Yield 90%, m.p.240^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.82(s, 3H, OCH_3), 4.13(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.5(s, 1H, =CH), 11.19 (s, 1H, NH), 11.57(s,1H, NH); ^{13}C NMR(75MHz, DMSO) δ: 42.7 (CH_2), 56.0(OCH_3), 113.6, 114, 122, 130.0, 136, 146.8, 168.3(C=O), 183 (C=S); Anal. Calcd. for $C_{22}H_{16}ClN_5O_2S_2$; C, 54.82; H, 3.35; N, 14.53%. Found: C, 54.40 ; H, 3.15; N, 14.01%.

5-{[6-(4-bromophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5b)

Yellow solid (Chloroform), Yield 95%, m.p.251^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(s, 3H, OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.52(s, 1H, =CH), 11.18 (s, 1H, NH), 11.47(s, 1H, NH); Anal. Calcd. for $C_{22}H_{16}BrN_5O_2S_2$; C, 50.19; H, 3.06; N, 13.30%. Found: C, 50.40 ; H, 3.15; N, 13.01%.

5-{[2-(4-methoxybenzyl)-6-phenylimidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5c)

Yellow solid (Chloroform), Yield 89%, m.p.276^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(s, 3H, OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 9H, Ar-H), 8.52(s, 1H, =CH), 11.18 (s, 1H, NH), 11.47(s, 1H, NH); ^{13}C NMR(75MHz, DMSO) δ: 42.7(CH_2), 56.0(OCH_3), 114.0, 122, 122.7, 130.2, 136.5, 146.8, 159.0, 163.7, 168.3(C=O), 183 (C=S); Anal. Calcd. for $C_{22}H_{17}N_5O_2S_2$; C, 59.04; H, 3.83; N, 15.65%. Found: C, 59.10 ; H, 3.65; N, 15.41%.

5-{[6-(4-methylphenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5d)

Yellow solid (Chloroform), Yield 92%, m.p.268^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 2.35(s, 3H, CH_3) 3.83(s, 3H, OCH_3) ,4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.45(s, 1H, =CH), 11.06 (s, 1H, NH), 11.27(s, 1H, NH); Anal. Calcd. for $C_{23}H_{19}N_5O_2S_2$; C, 59.85; H, 4.15; N, 15.17%. Found: C, 59.40 ; H, 4.05; N, 15.01%.

5-{[6-(4-methoxyphenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5e)

Yellow solid (Chloroform), Yield 90%, m.p.290^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(d, 6H, OCH_3), 4.23(s, 2H, CH_2), 6.92-7.84(m, 8H, Ar-H), 8.55(s, 1H, =CH), 11.06 (s, 1H, NH), 11.27(s, 1H, NH); Anal. Calcd. for $C_{23}H_{19}N_5O_3S_2$; C, 57.85; H, 4.01; N, 14.66%. Found: C, 59.40 ; H, 4.05; N, 15.01%.

5-{[6-(4-nitrophenyl)-2-(4-methoxybenzyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl]methylene}-2-thioxoimidazolidin-4-one (5f)

Yellow solid (Chloroform), Yield 95%, m.p.275^{0}C; IR (KBr) υ 3120, 3036, 2993, 1736, 1612, 1508 cm^{-1}; ^{1}H NMR(300MHz, DMSO) δ: 3.83(d, 6H, OCH_3), 4.42(s, 2H, CH_2), 6.92-7.84(m,8H, Ar-H), 8.55(s, 1H, =CH), 11.06 (s, 1H, NH), 11.27(s, 1H, NH); ^{13}C NMR(75MHz, DMSO): 42.7 (CH_2), 56.0(OCH_3), 114.0, 122, 127.9, 130.2, 136, 146.8, 159.0, 168.3(C=O),183(C=S); Anal. Calcd. for $C_{23}H_{16}N_6O_4S_2$; C, 53.65; H, 3.27; N, 17.06%. Found: C, 53.40 ; H, 3.14; N, 17.01%.

ANTIMICROBIAL ACTIVITY

ANTIBACTERIAL ACTIVITY

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922) and *Staphylococcus aureus* (ATTC-25923) bacterial stains by serial plate dilution method [13, 14]. Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 hours at 37°C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and placed in an incubator at 37°C for an hour. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each well labeled. A control was also prepared for the plates in the same way using DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Ciprofloxacin as standard. Zone of inhibition were determined and the results of such studies are summarized in table 1.

ANTIFUNGAL ACTIVITY

The newly synthesized compounds were screened for their antifungal activity against *Aspergillus flavus* (NCIM No.524), *Trichophyton mentagrophytes* (Recultured) in DMSO by serial plate dilution method. Sabouradus agar media was prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3ml saline to get a suspension of corresponding species. Agar media (20mL) was poured into each petri dish. Excess of suspension was decanted and the plated were dried by placing in an incubator at 37°C for an hour. Using an agar punch wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the same way using solvent DMSO. The petri dish were prepared in triplicate and maintained at 37°C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with Ciclopiroxol amine as the standard. Zone of inhibition were determined and the results are summarized in table 1.

Table 1:ANTIMICROBIAL ACTIVITY DATA

Comp. No.	R	MIC in µg/ml and Zone of inhibition (in mm)			
		Antibacterial Activity		Antifungal Activity	
		Escherichia coli	*Staphylococcus aureus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*
2a	Cl	6.25(16-20)	6.25(16-20)	25(<10)	25(<10)
2b	Br	25(<10)	6.25(16-20)	12.5(11-15)	11.5(11-15)
2c	H	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
2d	Me	25(<10)	25(<10)	25(<10)	6.25(16-20)
2e	OMe	12.5(11-15)	6.25(16-20)	6.25(16-20)	6.25(16-20)
2f	NO_2	6.25(16-20)	12.5(11-15)	25(<10)	12.5(11-15)
3a	Cl	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
3b	Br	25(<10)	25(<10)	6.25(16-20)	25(<10)
3c	H	6.25(16-20)	12.5(11-15)	6.25(16-20)	6.25(16-20)
3d	Me	6.25(16-20)	6.25(16-20)	12.5(11-15)	12.5(11-15)
3e	OMe	12.5(11-15)	6.25(16-20)	6.25(16-20)	6.25(16-20)
3f	NO_2	6.25(16-20)	6.25(16-20)	25(<10)	25(<10)
4a	Cl	25(<10)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4b	Br	12.5(11-15)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4c	H	6.25(16-20)	25(<10)	12.5(11-15)	12.5(11-15)
4d	Me	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
4e	OMe	6.25(16-20)	12.5(11-15)	6.25(16-20)	6.25(16-20)
4f	NO_2	6.25(16-20)	6.25(16-20)	6.25(16-20)	12.5(11-15)
5a	Cl	6.25(16-20)	12.5(11-15)	6.25(16-20)	6.25(16-20)
5b	Br	12.5(11-15)	6.25(16-20)	25(<10)	12.5(11-15)
5c	H	6.25(16-20)	6.25(16-20)	6.25(16-20)	6.25(16-20)
5d	Me	6.25(16-20)	6.25(16-20)	12.5(11-15)	12.5(11-15)
5e	OMe	6.25(16-20)	12.5(11-15)	25(<10)	6.25(16-20)
5f	NO_2	12.5(11-15)	12.5(11-15)	6.25(16-20)	25(<10)
Ciprofloxacin		6.25(16-20)	6.25(16-20)	-	-
Ciclopiroxol amine		-	-	6.25(16-20)	6.25(16-20)

Note: The MIC values were evaluated at concentration range 1.56-25μg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

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