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Research article

NOVEL ONE-POT THREE-COMPONENT SYNTHESIS OF NEW ETHYL 8-METHYL-3,6-DIPHENYL-2,6-DIHYDROPYRIMIDO[2,1-*B*][1,3,4]THIADIAZINE-7-CARBOXYLATE DERIVATIVES AND ITS ANTIMICROBIAL ACTIVITIES.

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ABSTRACT: A novel series of ethyl 8-methyl-3,6-diphenyl-2,6-dihydropyrimido[2,1-*b*][1,3,4]-thiadiazine-7-carboxylates **4** (**a-m**) were synthesized in good to excellent yield *via* cyclocondensation of 2-amino-5-pheny-6*H*-1,3,4-thiadiazine **1** (**a-d**) with ethyl acetoacetate (**2**) and various substituted aldehydes 3 (**a-d**) in presence of *p*-toluene sulfonic acid (PTSA) in acetonitrile. The structures of these compounds **4** (**a-m**) were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopic techniques and elemental analysis. All newly synthesized compounds **4** (**a-m**) were screened for antimicrobial activities.

Key words: One-Pot three-component synthesis, 2-amino-6*H*-1,3,4-Thiadiazines, PTSA, antimicrobial.

INTRODUCTION

Multi-component reactions (MCRs) are one-pot processes in which three or more reactants come together in a single reaction vessel to give a final product. MCRs is currently an important part of numerous research work involved in the drug discoveries to achieve synthetic targets in effective way, because they are easy to carry out, and provide rapid access to libraries of organic compounds with diverse substitution patterns. a) I Ugi et. al., 1994; b) I Ugi et. al., 2000; c) I Ugi et. al., 2001; d) C O Kappe, 2002 e) J Zhu and H Bienayne, 2005; f) K Kumaravel and G Vasuki, 2009]. These methods eliminate the isolation of intermediates, there by reduces the reaction time and increases the yield than the normal multistep methods (M Zhang et. al., 2006). Pyrimidine derivatives and heterocyclic annulated pyrimidines continue to attract the great interest because of their wide variety of biological activities such as anticancer (C R Petrie et.al., 1985), antiviral (M N Nasr and M M Gineinah, 2002) and anti-inflammatory activities (S M Sondhi, et. al., 2001). Pyrimido-[4,5-e][1,3,4] thiadiazines have the potential to exhibit distinct biological activities because of being nucleoside analogues (H Ogura, et.al., 1978). In 1893, Italian chemist Biginelli reported (P Biginelli, 1893) the simplest and most straightforward procedure involves three component one-pot cyclocondensation of the acetoacetic ester, aldehyde and third component as urea/thiourea in strong acidic condition to obtain a new compound 3,4-dihydropyrimidin-2(1*H*)-ones (or Biginelli compounds). Biginelli compounds like Pyrimidinones or dihydropyrimidinones (DHPMs) are biologically active and well known in the field of drug discovery. This stimulated an interest in the synthetic methods for their preparation chemical transformations and application. 1,3,4-thiadiazines represents the widely studied class of compounds among the six theoretically possible thiadiazine isomers; and are the most interest in a chemical sense because they are capable of undergoing intramolecular rearrangement to give thiazole and pyrazole derivatives. In addition 1,3,4-thiadiazines exhibit a broad spectrum of biological activites (S V Usolteva et. al., 1991) and in agriculture they act as a herbisides, pestisides, fungisides, insecticides, plant growth regulators, in photography and in dye manufacture.

The synthesis of some new fused tetrahydropyrimidino[4,5-e] thiadiazine was achieved (M S Chande et. al., 1999) by the reaction of thiocarbohydrazide with 5-bromo barbituric acid in presence of pyridine in ethanol. The obtained pyrimidino [4,5-e] thiadiazine was treated with different aldehydes afforded the corresponding Schiff's base. A simple one-pot synthesis of new 2-anilino-pyrimido[4,5-*e*][1,3,4] thiadiazines (M Bakavoli et. al., 2008) via an intermediates formed by the reaction of 5-bromo-2-chloro-6-methyl-4-(1-methylhydrazino) pyrimidine with various arylisothiocyanates in presence of triethylamine in boiling acetonitrile under nitrogen atmosphere.

Recently, a series of 5-alkyl-7-chloro-3-phenylazo-1-phenyl-1*H*-pyrimido[4,5-*e*] [1,3,4]thiadiazines were prepared (M Nikpour et.al., 2012) by condensation of the dithiazone with 5-bromo-2,4-dichloro-6-alkylpyrimidines in alkaline acetonitrile.

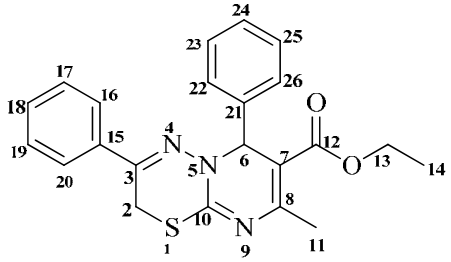
Literature survey reveals that, available methods for the synthesis of pyrimido-thiadiazine derivatives involve multistep reaction, exhaustive workup and poor yields. So here we communicate a novel method for the synthesis of new bicyclo heterocyclic compound containing 1,3,4-thiadiazine fused with pyrimidine moiety i.e., ethyl 8-methyl-3,6-diphenyl-2,6-dihydropyrimido[2,1-*b*][1,3,4] thiadiazine-7-carboxylates 4 (a-m).

MATERIALS AND METHODS

All chemicals/reagents were purchased from Merck Chemicals (India) and Fluka chemicals (India). The melting points were measured with micro melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellets on Shimadzu 8300 spectrometer. The ^{1}H NMR ($CDCl_3$) was recorded on a Agilent –NMR-vnrms 400 MHz spectrophotometer and ^{13}C NMR (DMSO-d_6) spectra were obtained on a Varian Gemini 400 MHz spectrometer. Chemical shifts are expressed in ppm (TMS was used as internal standard). Mass spectra were obtained on Agilent 6330 ion trap spectrophotometer and elemental analysis was performed on a Jusco microanalytical data unit. Thin layer chromatography (TLC) was performed on a pre-coated Silica Gel sheets (HF 254, Sd-fine) and visualization of the spots was done in iodine vapour and/or UV light. Chromatographic separations were carried out on silica gel (60-120) mesh using petroleum ether: acetone (9:1) as eluent.

General procedure for the synthesis of 2-amino-5-phenyl-6H-1,3,4-thiadiazine, **1a :** (P K Bose, 1924), (A P Novikova, 1921) The solution of phenacyl bromide (2.00g 10.00 mmol) was refluxed with thiosemicarazide (0.90g 10.00 mmol) in 20 ml of conc. HCl for about 30 minutes. After the completion of reaction (monitored through TLC), the reaction mixture was cooled to room temperature. The pale yellow solid thus obtained was filtered, washed with chloroform (3 X 10 mL), dried and recrystallized from methanol to give 2-amino-5-pheny-6*H*-1,3,4-thiadiazine **1a** in 70% yield.

Typical procedure for the synthesis of ethyl 8-methyl-3,6-diphenyl-2,6-dihydropyrimido[2,1-b][1,3,4] thiadiazine-7-carboxylate (4a).



2-amino-5-pheny-6*H*-1,3,4-thiadiazine (1a, 1.91g, 10.00 mmol), ethyl acetoacetate (2, 1.30g, 10.0 mmol), benzaldehyde (3a, 1.06g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) was dissolved in acetonitrile (30 mL) and the resulting solution was refluxed for about 2-3 hr. After the completion of reaction the reaction mixture was cooled to room temperature and extracted with $CHCl_3$ (3 X 25 mL), washed successively with water (2 x 25 mL), 2% dilute HCl solution and dried over anhydrous Na_2SO_4. The solvent was evaporated to give red viscous liquid, which was subjected to chromatographic separation (silica gel (60-120) using ethyl acetate and petroleum ether (2:8) as eluent, to get an orange colour solid ethyl 8-methyl-3,6-diphenyl-2,6-dihydropyrimido[2,1-*b*][1,3,4]thiadiazine-7-carboxylate (4a) in 75% yield (0.58g) m.p. 148-150ºC. IR (KBr, cm^{-1}): γ 2935 (C-H), 1610(C=N), 1740 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 6.94 Hz, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.75 (s, 2H CH_2), 4.22 (q, 2H, J = 7.2Hz, OCH_2), 6.41 (s, 1H, CH), 7.06-7.51 (m, 10H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.2 (C-14), 21.4 (C-11), 25.8 (C-2), 61.7 (C-13), 66.2 (C-6), 122.9 (C-7), 126.7 (C-24), 126.9 (C-26 and C-22), 128.2 (C-20 and C-16), 128.5 (C-23 and C-25), 128.9 (C-19 and C-17), 131.2 (C-18), 134.1 (C-15), 143.4 (C-21), 154.0 (C-8), 155.6 (C-3), 164.3 (C-10), 167.2 (C-12) ; MS for $C_{22}H_{21}N_3O_2S$: 392.15 $(MH)^+$; Anal. % Calcd: C: 67.50 H: 5.41 N: 10.73; Found: C: 67.40 H: 5.58 N: 10.57.

Ethyl 6-(4-chloro-phenyl)-8-methyl-3-phenyl-2,6-dihydropyrimido[2,1-b] [1,3,4]-thiadiazine-7-carboxylate (4b): Obtained from 2-amino-5-pheny-6*H*-1,3,4-thiadiazine (1a, 1.90g, 10.00 mmol), ethyl acetoacetate (2a, 1.30g, 10.00 mmol), 4-chloro-benzaldehyde (3b, 1.40g, 10.00 mmol) and PTSA (2.50g 15.00mmol) as red solid, yield 70% (2.98 g), m.p. 156-158ºC. IR (KBr cm^{-1}): γ 2940 (C-H), 1615 (C=N), 1735 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.28 (t, J = 6.94 Hz, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.70 (s, 2H CH_2), 4.33 (q, 2H, J = 7.2 Hz, OCH_2), 6.51 (s, 1H, CH), 7.28-7.62 (m, 9H, Ar-H). ^{13}C NMR (DMSO-d_6): δ 14.2 (C-14), 21.5 (C-11), 25.9 (C-2), 61.8 (C-13), 66.2 (C-6), 123.0 (C-7), 132.5 (C-24), 126.1 (C-26 and C-22), 128.2 (C-20 and C-16), 128. 7 (C-23 and C-25), 129.0 (C-17 and C-19), 131.3 (C-18), 134.1 (C-15), 141.6 (C-21), 154.1(C-8), 155.7 (C-3), 164.4 (C-10), 167.2 (C-12); MS for $C_{22}H_{20}ClN_3O_2S$: 426.21 $(MH)^+$. Anal. % Calcd: C: 62.04; H: 4.73; Cl: 8.32; N: 9.87. Found: C: 61.92; H: 4.88; N: 9.69.

Ethyl 8-methyl-3-phenyl-6-p-tolyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4c): Obtained from 2-amino-5-pheny-6*H*-1,3,4-thiadiazine (1a, 1.90g, 10.00 mmol), ethyl acetoacetate (2a, 1.30g, 10.00 mmol), 4-methyl-benzaldehyde (3c, 1.24g, 10.00 mmol) and PTSA (2.50g 15.00 mmol) as red solid, yield 68% (2.76g), m.p. 149-151ºC IR (KBr cm^{-1}): γ 2945 (C-H), 1618(C=N), 1733 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.23 (t, *J* = 6.94Hz, 3H, CH_3), 2.48 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.94 (s, 2H CH_2), 4.23 (q, 2H, *J* = 7.2 Hz, OCH_2), 6.51 (s, 1H, CH), 6.96-7.48 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.3 (C-14), 21.4 (CH_3), 21.4 (C-11), 25.8 (C-2), 61.7 (C-13), 66.2 (C-6), 122.9 (C-7), 136.5 (C-24), 126.8 (C-26 and C-22), 128.2 (C-20 and C-16), 128.9 (C-23 and C-25), 128.9 (C-17 and C-19), 131.2 (C-18), 134.1 (C-15), 140.3 (C-21),154.0 (C-8), 155.6 (C-3), 164.3 (C-10), 167.2 (C-12); MS for $C_{23}H_{23}N_3O_2S$: 406.11 $(MH)^+$; Anal. % Calcd: C: 68.12 H: 5.72 N: 10.36; Found: C: 68.09 H: 5.81 N: 10.21.

Ethyl 6-(4-methoxy-phenyl)-8-methyl-3-phenyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4d): Obtained from 2-amino-5-pheny-6*H*-1,3,4-thiadiazine (1a, 1.90g, 10.00 mmol), ethyl acetoacetate (2a, 1.30g, 10.00 mmol), 4-methoxy-benzaldehyde (3d, 1.36g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as reddish amorphous solid, yield 72% (3.03g). IR (KBr cm^{-1}): γ 2947 (C-H), 1620(C=N), 1730 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.25 (t, *J* = 6.94 Hz, 3H, CH_3), 3.74 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3), 4.14 (s, 2H CH_2), 4.22 (q, 2H, *J* = 7.2 Hz, OCH_2), 6.41 (s, 1H, CH), 6.89-7.48 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.2 (C-14), 21. 5 (C-11), 25.8 (C-2), 56.2 (-OCH_3), 61.7 (C-13), 66.3 (C-6), 122.8 (C-7), 158.8 (C-24), 125.9 (C-26 and C-22), 114.3 (C-23 and C-25), 128.2 (C-20 and C-16), 129.0 (C-17 and C-19), 131.3 (C-18), 134.2 (C-15), 136.7 (C-21), 154.1 (C-8), 155.7 (C-3), 164.4 (C-10), 167.2 (C-12); MS for $C_{23}H_{23}N_3O_3S$: 422.16 $(MH)^+$; Anal. % Calcd: C: 65.54 H: 5.50 N: 9.97; Found: C: 65.69 H: 5.34 N: 9.61.

Ethyl 3-(4-methoxy-phenyl)-8-methyl-6-phenyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4e) : Obtained from 2-amino-5-(4-methoxy-pheny)-6*H*-1,3,4-thiadiazine (1b, 2.21g, 10.00 mmol), ethyl aceto acetate (2a, 1.30g, 10.00 mmol), benzaldehyde (3a, 1.06g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as red solid, yield 72% (3.03g), m.p.135-137ºC : IR (KBr cm^{-1}): γ 2950 (C-H), 1615(C=N), 1728 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.27 (t, *J* = 6.9 Hz, 3H, CH_3), 3.83 (s, 3H, OCH_3), 2.46 (s, 3H, CH_3), 3.82 (s, 2H CH_2), 4.26 (q, 2H, *J*=7.2 Hz, OCH_2), 6.46 (s, 1H, CH), 6.93-7.51 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.3 (C-14), 21.5 (C-11), 25.8 (C-2), 55.9 (-OCH_3), 61.7 (C-13), 66.2 (C-6), 123.0 (C-7), 126.8 (C-24), 127.0 (C-26 and C-22), 128.6 (C-23 and C-25), 128.8 (C-20 and C-16), 114.4 (C-17 and C-19), 162.9 (C-18), 126.4 (C-15), 143.3 (C-21), 154.0 (C-8), 155.6 (C-3), 164.3 (C-10), 167.1 (C-12); MS for $C_{23}H_{23}N_3O_3S$: 422.10 $(MH)^+$; Anal. % Calcd: C: 65.54 H: 5.50 N: 9.97; Found: C: 65.72 H: 5.43 N: 9.64.

Ethyl 6-(4-chloro-phenyl)-3-(4-methoxy-phenyl)-8-methyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4f): Obtained from 2-amino-5-(4-methoxy-pheny)-6*H*-1,3,4-thiadiazine (1b, 2.21g, 10.00 mmol), ethyl aceto acetate (2a, 1.30ml, 10.00 mmol), 4-chloro-benzaldehyde (3b, 1.40g, 10.00 mmol) and PTSA (2.50g 15.00 mmoles) as yellow solid, yield 70% (3.19g), m.p.156-158ºC.IR KBr cm^{-1}): γ 2930 (C-H), 1617(C=N), 1726 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.26 (t, *J* = 6.94 Hz, 3H, CH_3), 3.90 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3), 4.24 (q, 2H, *J* = 7.2 Hz, OCH_2), 3.78 (s, 2H CH_2), 6.43 (s, 1H, CH), 6.90-7.53 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.3 (C-14), 21.5 (C-11), 25.8 (C-2), 55.9 (-OCH_3), 61.7 (C-13), 66.2 (C-6), 122.9 (C-7), 132.5 (C-24), 126.1 (C-26 and C-22), 128.7 (C-23 and C-25), 128.8 (C-20 and C-16), 114.4 (C-17 and C-19), 162.9 (C-18), 126.4 (C-15), 143.4 (C-21), 154.1 (C-8), 156.0 (C-3), 164.3 (C-10), 167.2 (C-12); MS for $C_{23}H_{22}ClN_3O_3S$: 456.14 $(MH)^+$. Anal. % Calcd: C: 60.59 H: 4.86 N: 9.22; Found: C: 60.66 H: 4.65 N: 9.14.

Ethyl 3,6-bis(4-methoxyphenyl)-8-methyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4g*):* Obtained from 2-amino-5-(4-methoxy-pheny)-6*H*-1,3,4-thiadiazine (**1b**, 2.21g, 10.00 mmol), ethyl aceto acetate (2a, 1.30ml, 10.00 mmol), 4-methoxy-benzaldehyde (3d, 1.36g, 10.00 mmol) and PTSA (2.5g, 15.00 mmol) as reddish pasty mass, yield 71% (3.21g). IR (KBr cm^{-1}): γ 2950 (C-H), 1615(C=N), 1728 (-C=O); ^{1}H NMR ($CDCl_3$): δ 1.28 (t, *J* = 6.94Hz, 3H, CH_3), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3), 4.24 (q, 2H, *J* = 7.2 Hz, OCH_2), 3.83 (s, 2H, CH_2), 6.48 (s, 1H, CH), 6.95-7.58 (m, 8H, Ar-H); ^{13}C NMR(DMSO-d_6): δ 14.3 (C-14), 21.5 (C-11), 25.9 (C-2), 55.9 & 55.9 (OCH_3), 61.7 (C-13), 66.2 (C-6), 122.9 (C-7), 158.8 (C-24), 125.9 (C-26 and C-22), 114.3 (C-23 and C-25), 128.4 (C-20 and C-16), 114.5 (C-17 and C-19), 162.9 (C-18), 126.4 (C-15), 136.7 (C-21), 154.2 (C-8), 155.7 (C-3), 164.3 (C-10) ,167.3 (C-12); MS for $C_{24}H_{25}N_3O_4S$: 452.14 $(MH)^+$; Anal. % Calcd: C: 63.84 H: 5.58 N: 9.31; Found: C: 63.91 H: 5.63 N: 9.24;

Ethyl 3-(4-chlorophenyl)-8-methyl-6-phenyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4h): Obtained from 2-amino-5-(4-chloro-pheny)-6*H*-1,3,4-thiadiazine (**1c,** 2.25g, 10.00 mmol), ethyl aceto acetate (**2a**, 1.30g, 10.00 mmol), benzaldehyde (3a, 1.06g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as yellow solid, yield 67% (2.85g), m.p.134-136ºC. IR (KBr cm^{-1}): γ 2935 (C-H), 1610(C=N), 1735 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.28 (t, *J* = 6.94 Hz, 3H, CH_3), 2.55 (s, 3H, CH_3), 4.27 (q, 2H, *J* = 7.2 Hz, OCH_2), 3.83 (s, 2H, CH_2), 6.48 (s, 1H, CH), 7.26-7.58 (m, 9H, Ar-H);

^{13}C NMR (DMSO-d_6): δ 14.3 (C-14), 21.4 (C-11), 25.8 (C-2), 61.8 (C-13), 66.3 (C-6), 123.1 (C-7), 126.7 (C-24), 126. 9 (C-26 and C-22), 128.5 (C-23 and C-25), 128.1 (C-20 and C-16), 129.0 (C-17 and C-19), 136.6 (C-18), 132.1 (C-15), 143.5 (C-21), 154.1(C-8), 155.6 (C-3), 164.4 (C-10), 167.2 (C-12); MS for $C_{22}H_{20}ClN_3O_2S$: 426.11 $(MH)^+$; Anal. % Calcd: C: 62.04 H: 4.73 N: 9.87; Found: C: 61.94 H: 4.68 N: 9.74.

Ethyl 3,6-bis(4-chlorophenyl)-8-methyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4i): Obtained from 2-amino-5-(4-chloro-pheny)-6*H*-1,3,4-thiadiazine (**1c,** 2.25g, 10.00 mmol), ethyl aceto acetate (**2a**, 1.32ml, 10. 00 mmol), 4-chloro-benzaldehyde (**3b,** 1.40g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as bright yellow solid, yield 70%, (3.22g), m.p.142-144ºC; **IR** (KBr cm^{-1}): γ 2928 (C-H), 1615 (C=N), 1724 (C=O); **^{1}H NMR ($CDCl_3$):** δ 1.26 (t, *J* = 6.94 Hz, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 7.2 Hz, OCH_2), 3.86 (s, 2H, CH_2), 6.43 (s, 1H, CH), 7.21-7.52 (m, 8H, Ar-H); **^{13}C NMR(DMSO-d_6):** δ 14.3 (C-14), 21.5 (C-11), 25.9 (C-2), 61.9 (C-13), 66.3 (C-6), 123.1 (C-7), 132.5 (C-24), 126.1(C-26 and C-22), 128.7 (C-23 and C-25), 128.2 (C-20 and C-16), 129.0 (C-17 and C-19), 136.65 (C-18), 132.2 (C-15), 141.2 (C-21), 154.0 (C-8), 155.7 (C-3), 164.4 (C-10) , 167.25 (C-12); **MS** for $C_{22}H_{19}Cl_2N_3O_2S$**:** 460.31 $(MH)^+$; Anal. % Calcd: C: 57.40 H: 4.16 N: 9.13; Found**:** C: 57.26 H: 4.08 N: 9.24.

Ethyl 3-(4-chlorophenyl)-8-methyl-6-p-tolyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4j)*:* Obtained from 2-amino-5-(4-chloro-pheny)-6*H*-1,3,4-thiadiazine (1c. 2.25g, 10.00 mmol), ethyl aceto acetate (2a, 1.30g, 10.00 mmol), 4-methyl-benzaldehyde (3c, 1.20g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol). Pale yellow solid, yield 74% (3.25g), m.p. 147-149ºC. IR (KBr cm^{-1}): γ 2930 (C-H), 1620 (C=N), 1728 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.25 (t, *J* = 6.94 Hz, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.51(s, 3H, CH_3), 4.26 (q, 2H, *J* = 7.2 Hz, OCH_2), 3.89 (s, 2H, CH_2), 6.51 (s, 1H, CH), 7.26-7.54 (m, 8H, Ar-H); ^{13}C NMR(DMSO-d_6): δ 14.3 (C-14), 21.4 (C-11), 21.5 (-CH_3) 25.9 (C-2), 61.8 (C-13), 66.3 (C-6), 123.0 (C-7), 136.5 (C-24), 126.8 (C-26 and C-22), 128.9 (C-23 and C-25), 128.2 (C-20 and C-16), 128.9 (C-17 and C-19), 136.6 (C-18), 132.1 (C-15), 142.5 (C-21), 154.1 (C-8), 155.7 (C-3), 164.3 (C-10), 167.20 (C-12); MS for $C_{23}H_{22}ClN_3O_2S$: 440.11 $(MH)^+$; Anal. % Calcd: C: 62.79 H: 5.04 N: 9.55; Found: C: 62.64 H: 5.93 N: 9.61.

Ethyl 8-methyl-3-(4-Nitrophenyl)-6-phenyl-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4k): Obtained from 2-amino-5-(4-Nitro-pheny)-6*H*-1,3,4-thiadiazine (1d, 2.36g, 10.00 mmol), ethyl aceto acetate (2a, 1.30g, 10.00 mmol), benzaldehyde (3a, 1.064g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as pale yellow solid, yield 66 (2.88g)%, m.p. 162-164ºC. IR (KBr cm^{-1}): γ 2938 (C-H), 1718 (-C=O), 1616 (C=N); ^{1}H **NMR ($CDCl_3$):** δ 1.28 (t, J = 6.94 Hz, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.27 (q, 2H, *J* = 7.2 Hz, OCH_2), 4.14 (s, 2H, CH_2), 6.61(s, 1H, CH), 7.08-7.95 (m, 9H, Ar-H). ^{13}C NMR(DMSO-d_6): δ 14.3 (C-14), 21.5 (C-11), 25.9 (C-2), 61.9 (C-13), 66.3 (C-6), 123.1 (C-7), 126.6 (C-24), 126.9 (C-26 and C-22), 128.5 (C-23 and C-25), 127.8 (C-20 and C-16), 127.2 (C-17 and C-19), 151.2 (C-18), 140.2 (C-15), 143.4 (C-21),154.3 (C-8), 155.7 (C-3), 164.4 (C-10), 167.32 (C-12); MS for $C_{22}H_{20}N_4O_4S$: 437.15 (MH^+); Anal. % Calcd: C: 60.54 H: 4.62 N: 12.84. Found: C: 60.40 H: 4.48 N: 12.52.

Ethyl6-(4-chloro-phenyl)-8-methyl-3-(4-Nitro-phenyl)-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4l): Obtained from 2-amino-5-(4-Nitro-pheny)-6*H*-1,3,4-thiadiazine (1d, 2.36g, 10.00 mmol), ethyl aceto acetate (2a, 1.30ml, 10.00 mmol), 4-chloro-benzaldehyde (3b, 1.40g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as reddish solid, yield 68% (3.20g), m.p.154 -156ºC. IR (KBr cm^{-1}): γ 2934 (C-H), 1626 (C=N), 1736 (C=O); ^{1}H NMR ($CDCl_3$-d_3): δ 1.27 (t, *J* = 6.94 Hz, 3H, CH_3), 2.57 (s, 3H, CH_3), 4.27 (q, 2H, *J* = 7.2 Hz, OCH_2), 4.17 (s, 2H, CH_2), 6.54 (s, 1H, CH),7.04-7.95 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 14.4 (C-14), 21.5 (C-11), 25.9 (C-2), 61.8 (C-13), 66.3 (C-6), 123.1 (C-7), 132.5 (C-24), 126.1 (C-26 and C-22), 128. 7 (C-23and C-25), 127.9 (C-20 and C-16), 127.2 (C-17 and C-19), 151.2 (C-18), 140.2 (C-15), 141.5 (C-21), 154.0 (C-8), 155.6 (C-3), 164.5 (C-10), 167.26 (C-12); MS for $C_{22}H_{19}ClN_4O_4S$: 471.10 $(MH)^+$; Anal. % Calcd: C: 56.11 H: 4.07 N: 11.90; Found: C: 55.94 H: 4.17 N: 11.61.

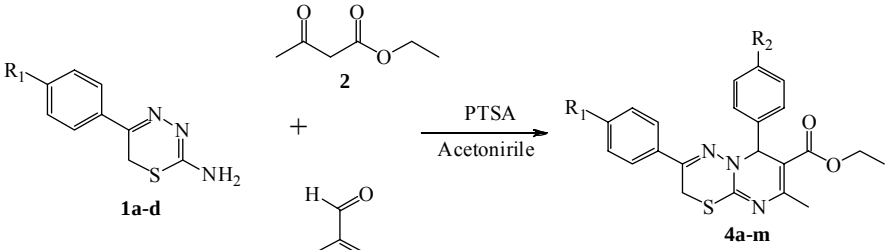
Ethyl 6-(4-methox-yphenyl)-8-methyl-3-(4-Nitro-phenyl)-2,6-dihydropyrimido[2,1-b][1,3,4]-thiadiazine-7-carboxylate (4m): Obtained from 2-amino-5-(4-Nitro-pheny)-6*H*-1,3,4-thiadiazine (1d, 2.36g, 10.00 mmol), ethyl aceto acetate (2a, 1.30g, 10.00 mmol), 4-methoxy-benzaldehyde (3d, 1.36g, 10.00 mmol) and PTSA (2.50g, 15.00 mmol) as yellowish red gummy mass, yield 70% (3.26g). IR (KBr cm^{-1}): γ 2925 (C-H), 1625 (C=N), 1720 (C=O); ^{1}H NMR ($CDCl_3$): δ 1.23 (t, *J* = 6.94 Hz, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.27 (q, 2H, *J* = 7.2Hz, OCH_2), 4.21 (s, 2H, CH_2), 6.51 (s, 1H, CH), 7.02-8.05 (m, 8H, Ar-H); ^{13}C NMR ($CDCl_3$-d_6): δ 14.3 (C-14), 21.6 (C-11), 25.9 (C-2), 55.9 (-OCH_3), 61.8 (C-13), 66.2 (C-6), 122.9 (C-7), 158.8 (C-24), 125.8 (C-26 and C-22), 114.3 (C-23 and C-25), 127.9 (C-20 and C-16), 127.2 (C-17 and C-19), 151.2 (C-18), 140.2 (C-15), 135.7 (C-21), 154.0 (C-8), 155.7 (C-3), 164.4 (C-10), 167.8 (C-12); MS: 467.14 $(MH)^+$; Anal. % Calc for $C_{23}H_{22}N_4O_5S$: C: 59.22 H: 4.75 N: 12.00; Found: C: 59.04 H: 4.57 N: 11.81;

Antimicrobial activity

The newly synthesized compounds 4 (a-m) were screened for *in vitro* anti-bacterial activity against *Bacillus cereus* (MTCC 8372), *Staphylococcus aureus* (MTCC 96) (gram positive bacteria) *Escherichia coli* (MTCC 724) *and Klebsiella pneumonia*, (gram negative *bacteria*) using the agar disc diffusion method. (J M Andrews, 2008). The compounds 4 (a-m) were dissolved in dimethylformamide (DMF) at the concentration 50 and 100µg/mL and placed on the inoculated plates, after allowing at 4ºC for 2h, they were incubated at 37ºC for 24h. The inhibition zone was measured in millimetres. Tetracycline was used as the standard drug. In addition *in vitro* antifungal screening (J R Zgoda and J R Porter, 2001) of the synthesized compounds 4 (a-m) was carried out against *Aspergillus flavus* (MTCC 873), *Aspergillus niger* (MTCC 281), *Fusarium oxysporum* (MTCC 284), and *Fusarium monaliforme* (MTCC 156) using Nystatin as standard drug. The micro dilution method was used to evaluate the minimum inhibitory concentration (MIC) of all the synthesized compounds as summarized in Table-1. The compounds were stable in the Nutrient agar and Potato dextrose agar. The MIC for fungal strains was performed using 96-well plate. The fungi were maintained on potato dextrose agar (PDA) medium at 28ºC. Six replicate determinations were performed for all the compounds and the results were taken as a mean of at least three determinations.

RESULTS AND DISSCUSSION

The synthesis of ethyl 8-methyl-3,6-diphenyl-2,6-dihydropyrimido [2,1-*b*][1,3,4] thiadiazine-7-carboxylate derivatives 4 (a-m) in excellent yield was achived by the cyclocondensation reaction of 2-amino-6*H*-1,3,4-thiadiazines[14,15] 1 (a-d) with ethyl acetoacetate (2) and various substituted aldehydes 3 (a-d) in presence of catalytic amount of PTSA (T Jin, S Zhang and T Li 2002), in acetonitrile (Scheme 1).

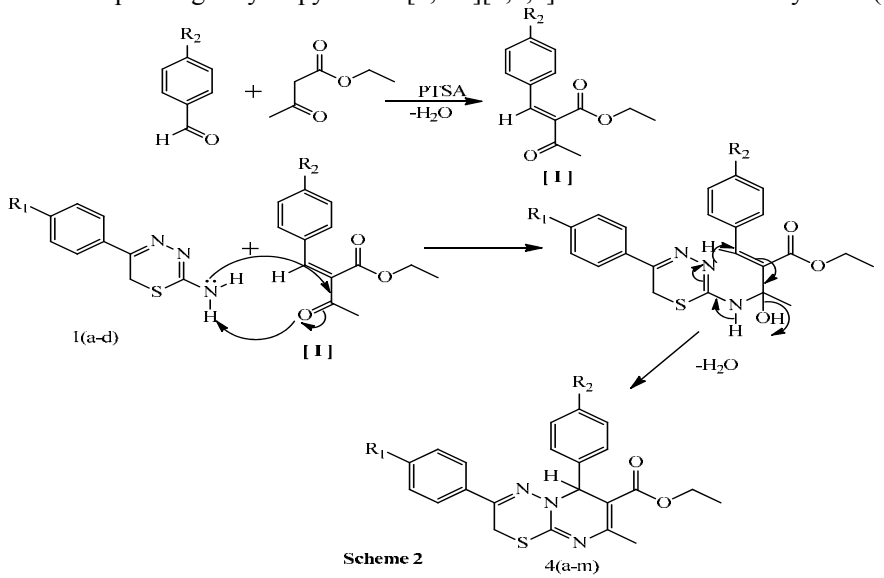


1a : R_1 = H,
1b : R_1 = *p*-OCH_3-,
1c : R_1 = *p*-Cl,
1d : R_1 = *p*-NO_2-
3a : R_2 = H,
3b : R_2 = *p*-Cl,
3c : R_2= *p*-CH_3-,
3d : R_1 = *p*-OCH_3-

4a : R_1 = H, R_2 = H,
4b : R_1 = H, R_2 = *p*-Cl,
4c : R_1 = H, R_2 = *p*-CH_3-,
4d : R_1 = H, R_2 = *p*-OCH_3-,
4e : R_1 = *p*-OCH_3-, R_2 = H,
4f : R_1 = *p*-OCH_3-, R_2 = *p*-Cl,
4g : R_1 = *p*-OCH_3-, R_2 = *p*-OCH_3-,
4h : R_1 = *p*-Cl-, R_2 = H,
4i : R_1 = *p*-Cl-, R_2 = *p*-Cl-,
4j : R_1 = *p*-Cl-, R_2 = *p*-CH_3-,
4k : R_1 = *p*-NO_2-, R_2 = H,
4l : R_1 = *p*-NO_2-, R_2 = *p*-Cl,
4m : R_1= *p*-NO_2-,R_2 = *p*-OCH_3-,

Scheme 1

The tentative mechanism for the above reaction is as shown in Scheme 2. Initially the in situ preparation of intermidate [1] was achieved by the reaction of substituted aldehydes with ethyl acetoacetate in presence of PTSA. Further 2-amino-6*H*-1,3,4-thiadiazine 1 (a-d) reacts with intermediate [1] with the elimination of water molecule to yield the corresponding dihydropyrimido [2,1-*b*][1,3,4] thiadiazine-7-carboxylate 4 (a-m).



Scheme 2

The newly synthesized compounds were characterized by their IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis. For instance, the IR spectra of 4 (a-m) showed the stretching vibration bands at around 2925-2950 cm^{-1} corresponding to presence of -CH- group, and a vibration band 1718-1740 cm^{-1} indicates the presence of >C= O group in the compound. The ^{1}H NMR spectra of compounds 4 (a-m) in showed, the signals due to C–CH_3 proton in the region δ 2.40-2.61 ppm, multiplet peaks of ester group, like quatret peak appeared in the region δ 4.21-4.27 ppm due to-CH_2-CH_3, and triplet peak due to –CH_2-CH_3 appeared at δ 1.23-1.28ppm, while singlet peak of -CH- group appeared in the region δ 6.41-6.62 ppm and aromatic protons at δ 6.89-8.10 ppm. Absence of singlet peak of –NH_2 in the region δ 8.50-9.10 ppm confirms the formation of condensed adduct. In ^{13}C NMR spectra presence of additional peaks in the range of δ 14.2- 14.4 ppm (C-14), 21.4-21.6 ppm (C-11), 61.7-61.9 ppm (C-13), 66.2-66.3 (C-6) was observed. All synthesized compounds 4 (a-m) showed MH+ as a base peak in the mass spectra.

Table 1: The minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) in µg/mL of synthesized compounds against tested strains.

Compds	Antibacterial activity								Antifungal activity							
	Gram positive				Gram negative											
	B. cereus		*S. aureus*		*E. coli*		*K. pneumonia*		*A. flavus*		*A. niger*		*F. oxysporum*		*F. monaliforme*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
4a	85	280	80	260	85	250	90	265	65	250	55	265	60	270	75	280
4b	60	230	80	255	80	260	85	250	**25**	**135**	**10**	**120**	**20**	**125**	**15**	**120**
4c	60	250	45	255	55	260	50	240	60	290	65	285	50	280	60	275
4d	**10**	**130**	**15**	**135**	**15**	**140**	**15**	**135**	**10**	**125**	**20**	**120**	**20**	**130**	**15**	**145**
4e	35	195	50	215	35	220	45	220	35	215	40	220	45	210	50	215
4f	**20**	**150**	**25**	**165**	40	210	35	195	45	235	50	220	40	210	55	230
4g	**40**	**120**	**15**	**125**	**10**	**120**	**15**	**130**	**30**	**190**	**25**	**175**	**20**	**165**	**15**	**120**
4h	80	280	95	275	85	285	90	270	75	265	85	295	70	280	90	300
4i	75	290	90	295	85	280	80	295	95	290	85	280	65	270	70	295
4j	65	280	85	290	90	275	80	270	**25**	**135**	**30**	**155**	**20**	**140**	**45**	**165**
4k	80	280	90	270	95	285	85	280	85	270	80	280	65	245	**25**	**115**
4l	**30**	**180**	**35**	**195**	85	295	70	280	90	285	95	275	50	280	55	260
4m	95	270	80	275	70	265	65	250	80	275	75	260	80	270	65	285
Tetracycline	**5**	**120**	**10**	**120**	**12**	**120**	**8**	**120**	---	---	---	---	---	---	---	---
Nystatin	---	---	---	---	---	---	---	---	**08**	**100**	**10**	**100**	**15**	**100**	**12**	**100**

[a](Mean six replicate ± standard deviation).

Structure activity relationship:

The novel series of 4 (a-m) contains pyrimidine moiety (which is one of the nucleosidic base) and hence a good antimicrobial activity was expected, the results revealed that, compounds 4d, 4f, 4g and 4l exhibit good to potent antimicrobial activity. Of the five tested bacterial strains, gram-positive bacteria were inhibited mostly by compounds 4d, 4f and 4g which containsOCH_3 group along with Cl on the para position of the phenyl ring. While the gram-negative bacteria were inhibited by compound 4g which contains only OCH_3 group at the para position of both the phenyl rings of thiadiazine and pyrimidine moiety. Compound 4d and 4g, showed excellent antimicrobial activity against all the tested strains of microbes, this may be due to the presence of OCH_3 group on both the phenyl rings of thiadiazine as well as pyrimidine moieties. The compound 4l containing NO_2 do not contribute much to the antimicrobial activity against both the strains. With these above findings we conclude that the presence of electron withdrawing group like NO_2 group is not effective enough to inhibit the growth of microbes. The compounds 4b and 4j containing –Cl group were less active against bacterial strains but they possess good antifungal activity. While the compounds 4e and 4f showed moderate activity. Except the compound 4k, the remaining compounds showed less activity against *Fusarium monaliforme.*

CONCLUSION

In conclusion, we have achieved the synthesis of novel bicyclic pyrimido thiadiazine derivatives 4 (a-m) through an efficient one pot three component chemical transformation. All the synthesized compounds 4 (a-m) have been investigated for their *in vitro* antimicrobial activity. Accordingly, these novel series of bicyclo pyrimido thiadiazine analogues emerged as a potent antibacterial and antifungal agents. Among the synthesized compounds, 4g showed excellent antimicrobial activity in comparison with standard drug. Hence, it could be a promising drug candidate for microbial infections.

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