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Bicyclic [1,3,4]Thiadiazolo[3,2-α]Pyrimidine Analogues: Novel One-Pot Three-Component Synthesis, Antimicrobial, and Antioxidant Evaluation

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Abstract: A novel one-pot three-component synthesis of 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-α)pyrimidine-6-yl)ethanone (4a-i) derivatives *via* cyclo-condensation of substituted 2-amino-[1,3,4]thiadiazole (1a-c), acetylacetone (2) and various aromatic aldehydes (3a-c) in the presence of *p*-toluene sulfonic acid (PTSA) in acetonitrile. Spectral data and elemental analysis have characterized the newly synthesized compounds. The new analogs were screened for their antibacterial and antifungal activities. The majority of the tested compounds displayed significant to moderate efficacy against most of the designated organisms. Among the tested compounds, 4b, 4e, and 4h showed noteworthy efficacy against selected microbes, and compounds 4c and 4i were found to be exceptionally efficient against selected fungal strains. Compound 4c, 4e, 4f, 4i were also designated as best antioxidants against NOx.

Keywords: one-pot three-component synthesis; 1,3,4-thiadiazolo[3,2-α]pyrimidine; antibacterial activity.

1. Introduction

Multi-component reactions [1] are one-pot processes in which three or more reactants come together in a single reaction vessel to give a final product. The simplest and most straightforward procedure involves three-component one-pot cyclo-condensation [2] of the acetoacetic ester, aldehyde, and third component as urea/thiourea [3] in strong acidic condition to give biologically active Biginelli compounds [4] like pyrimidinones or 3,4-dihydropyrimidine-2(1*H*)-ones. Zhao and co-workers [5] synthesized the novel ethyl 7-methyl 2,5-diphenyl-5*H*-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-carboxylate derivatives through one-pot three-component chemical transformation by microwave irradiation using acetic acid without any catalyst. Synthesis of 1,3,4-thidiazolo-(3,2-α)pyrimidine-6-carbonitrile derivatives was achieved [6] using optimized reaction conditions. It showed good binding mode in the active site of STAT3 enzyme inhibitors [7] to treat breast cancer [8]. The cytotoxic activity [9] was found in 2-alkanesulfinyl/alkanesulfonyl-7-methyl-5*H*-1,3,4-thiadiazolo[3,2-α]pyrimidin-

5-one derivatives [10], especially the strong activity was shown by the compounds which have electrophilic substituent group on the 2-position such as alkyl sulfoxide or alkyl sulfone.

New series of biologically active sulfonamide derivatives [11] of [1,3,4]thiadiazolo[3,2-α]pyrimidine were synthesized and investigated for their antitumor activity. Some of them were tested for their *in vitro* and *in vivo* antitumor activities. Abdel Rahman and co-workers [12] synthesized the substituted imidazo[2,1-β]-1,3,4-thiadiazoles, substituted 1,3,4-thiadiazolo[3,2-α]pyrimidines and 1,3-disubstituted thiourea. Most of the compounds exhibited potent cytotoxic activity against tumor cell line A549 (non-small cell lung cancer cell line) [13] using sulforhodamine B (SRB) standard method [14]. Recently, Nagaraju and co-workers reported green synthesis [15] and characterization of novel [1,3,4]thiadiazolo/benzo[4,5]thiazolo[3,2-a]pyrimidines *via* the multi-component reaction between chosen substrates of 1,3,4-thiadiazole-amines or 2-amino-benzothiazole, aldehydes and active methylene compounds in ethanol solvent at room temperature using vanadium oxide loaded on fluorapatite as a robust and sustainable catalyst. Recently, 7-oxo-7*H*-[1,3,4]thiadiazolo[3,2-a]pyrimidine-5-carboxylate derivatives were conveniently synthesized under mild condition through regioselective cycloaddition reactions [16].

The thiadiazolo-pyrimidine nucleus and its substituted products, as well as several other substances, belongs to the pseudo purine class, were reported to have interesting biological profiles, including the antiviral [17-18], anti-cancer [19], antibiofilm [20], antitumor [21], antitubercular and antibacterial [22] activities. In last few decades, these analogues were synthesized as PARP1 Inhibitors [23], STAT3 Inhibitor [24], anticancer [25], antiglycation [26] and antioxidant [27]. This observation draws our attention to develop the synthesis of some bicycle heterocyclic compounds containing 1,3,4-thiadiazine fused with pyrimidine moiety, i.e., 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-α)pyrimidine-6-yl)ethanone (4a-i) derivatives and their antimicrobial and antioxidant studies.

2. 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 an Agilent–NMR-vnrms 400 MHz spectrometer and ^{13}C NMR (DMSO-d_6) spectra were obtained on a Varian Gemini 400 MHz spectrometer. The chemical shifts are expressed in ppm (TMS was used as an internal standard). Mass spectra were obtained on Agilent 6330 ion trap spectrophotometer, and elemental analysis was performed on a Jusco micro-analytical data unit. TLC was performed on pre-coated silica gel sheets (HF 254, TLC was performed on pre-coated Silica Gel sheets (HF 254, Sd-fine), and visualization of the spots was done in iodine vapor and/or UV light. Chromatographic separation was carried out on silica gel (60-120) mesh using petroleum ether: acetone (9:1) as eluent.

2.1. General procedure for the synthesis of 2-amino-5-phenyl-1,3,4-thiadiazole (1a).

A mixture of benzaldehyde (1.06g, 10.00mmol), thiosemicarbazide hydrochloride (1.40g, 15.00mmol) in 20 ml of ethanol was refluxed for about 30 minutes. After completing the reaction, the reaction mixture was cooled to room temperature, filtered, and dried to get solid white thiosemicarbazones. The obtained thiosemicarbazones were oxidized with 10%

ferric chloride solution (10 ml) in the presence of ethanol (30ml) for about 30 minutes to give yellow solid, which was filtered, extracted with chloroform (3 x 20 ml), washed with water, dried and recrystallized from ethanol to give cyclized pale yellow solid 2-amino-5-phenyl-1,3,4-thiadiazole [28] 1a in 80% yield (1a, 1.41g).

2.2. Typical procedure for the synthesis of 1-(7-methyl-2,5-diphenyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4a).

A mixture of acetylacetone (2.00g, 20.00mmol) and benzaldehyde (3a, 1.06g, 10.00mmol) in acetonitrile (25 ml) was refluxed on a water bath in the presence of acidic PTSA (2.50g, 15.00mmol) for about 30 minutes. Meanwhile, the 2-amino-5-phenyl-1,3,4-thiadiazole (1a, 1.77g, 10.00mmol) was added to the above reaction mixture and again refluxed for about 50-60 minutes. After the completion of the reaction, the reaction mixture was cooled to room temperature, extracted with chloroform (3 x 25 ml), washed with water (2 x 25 ml) and 2% dilute HCl solution, finally dried over anhydrous Na_2SO_4. The solvent was evaporated to give yellow viscous solid, which was subjected to column chromatographic technique (silica gel 60-120 mesh) using ethyl acetate and petroleum ether (2:8) as eluent to get an pale yellow solid 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-*α*)pyrimidine-6-yl)ethanone 4a in 80% yield (2.77g); m.p. 122-124ºC. IR (KBr, cm^{-1}): γ 2972 (C-H), 1728 (>C=O), 1610 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 2.27 (s, 3H, -$COCH_3$), 2.96 (s, 3H, -CH_3), 4.60 (s, 1H, -CH-), 7.23-7.80 (m, 10H, Ar-H); ^{13}C NMR (DMSO-*d6*): *δ* 21.2 (C-10), 27.4 (C-12), 64.6 (C-5), 126.5 (C-22), 127.0 (C-20 and C-24), 128.6 (C-21 and C-23), 129.0 (C-15 and C-17), 129.6 (C-14 and C-18), 130.8 (C-13), 131.2 (C-16), 132.2 (C-6), 143.4 (C-19), 143.8 (C-2), 151.8 (C-7), 160.4 (C-9), 196.6 (C-11); MS for $C_{20}H_{17}N_3OS$: 347.12 (MH^+, 100%), 348.12 (MH^{+1}, 24.6%), 349.14 (MH^{+2}, 6.2%); Elemental analysis: Calculated: C, 69.12; H, 4.95; N, 12.10; Found: C, 69.10; H, 4.92; N, 12.08.

2.3. 1-(5-(4-methoxyphenyl)-7-methyl-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4b).

Obtained from 2-amino-5-phenyl-1,3,4-thiadiazole (1a, 1.77g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 85% (3.20), m.p. 138-140ºC. IR (KBr, cm^{-1}): γ 2952 (C-H), 1730 (>C=O), 1620 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 2.24 (s, 3H, -$COCH_3$), 2.98 (s, 3H, -CH_3), 4.62 (s, 1H, -CH-), 7.32-7.80 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d6*): *δ* 21.0 (C-10), 27.2 (C-12), 56.4 (-OCH_3), 64.2 (C-5), 114.6 (C-21 and C-23), 126.2 (C-20 and C-24), 128.6 (C-15 and C-17), 129.4 (C-14 and C-18), 130.8 (C-13), 131.2 (C-16), 132.2 (C-6), 136.4 (C-19), 144.2 (C-2), 151.4 (C-7), 160.6 (C-9), 196.8 (C-11); MS for $C_{21}H_{19}N_3O_2S$: 377.12 (MH^+, 100%), 378.12 (MH^{+1}, 24.6%), 379.14 (MH^{+2}, 5.8%); Elemental analysis: Calculated: C, 66.80; H, 5.08; N, 11.14; Found: C, 66.78; H, 5.02; N, 11.12.

2.4. 1-(5-(4-chlorophenyl)-7-methyl-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4c).

Obtained from 2-amino-5-phenyl-1,3,4-thiadiazole (1a, 1.77g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 82% (3.14g), m.p. 152-154ºC. IR (KBr, cm^{-1}): γ 2950 (C-H), 1738 (>C=O), 1615 (C=N) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 2.26 (s, 3H, -$COCH_3$), 2.89

(s, 3H, -CH_3), 4.66 (s, 1H, -CH-), 7.30-7.74 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d6*): δ 21.4 (C-10), 27.0 (C-12), 64.4 (C-5), 134.4 (C-22), 126.8 (C-20 and C-24), 128.4 (C-21 and C-23), 129.2 (C-15 and C-17), 129.4 (C-14 and C-18), 130.6 (C-13), 131.6 (C-16), 132.0 (C-6), 142.4 (C-19), 144.0 (C-2), 152.2 (C-7), 160.6 (C-9), 196.8 (C-11); MS for $C_{20}H_{16}ClN_3OS$: 381.10 (MH^+, 100%), 383.12 (MH^{+1}, 383.2%), 382.08 (MH^{+2}, 24.4%); Elemental analysis: Calculated: C, 62.90; H, 4.24; Cl, 9.28; N, 11.00; Found: C, 62.88; H, 4.20; Cl, 9.26; N, 10.80

2.5. 1-(2-(4-methoxyphenyl)-7-methyl-5-phenyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4d).

Obtained from 5-(4-methoxyphenyl)-1,3,4-thiadiazole-2-amine (1b, 2.07g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) benzaldehyde (3a, 1.06g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 86% (3.24g), m.p. 144-146^{o}C. IR (KBr, cm^{-1}): γ 2970 (C-H), 1744 (>C=O), 1518 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H, -$COCH_3$), 2.92 (s, 3H, -CH_3), 3.84 (s, 3H, -OCH_3), 4.64 (s, 1H, -CH-), 7.02-7.86 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d6*): δ 21.6 (C-10), 27.2 (C-12), 56.4 (-OCH_3), 64.2 (C-5), 114.6 (C-15 and C-17), 123.2 (C-13), 126.8 (C-22), 127.2 (C-20 and C-24), 128.8 (C-21 and C-23), 130.4 (C-14 and C-18), 132.2 (C-6), 143.4 (C-19), 144.2 (C-2), 152.2 (C-7), 160.0 (C-9), 163.0 (C-16), 196.8 (C-11); MS for $C_{21}H_{19}N_3O_2S$: 377.46 (MH^+, 100%), 378.10 (MH^{+1}, 25%), 379.12 (MH^{+2}, 6.2%); Elemental analysis: Calculated: C, 66.80; H, 5.06; N, 11.12; Found: C, 66.78; H, 5.00; N, 11.10.

2.6. 1-(2,5-bis(4-methoxyphenyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4e).

Obtained from 5-(4-methoxyphenyl)-1,3,4-thiadiazole-2-amine (1b, 2.07g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 80% (3.25g), m.p. 140-142^{o}C. IR (KBr, cm^{-1}): γ 2955 (C-H), 1728 (>C=O), 1615 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, -$COCH_3$), 2.96 (s, 3H, -CH_3), 3.84-3.88 (s, 6H, -OCH_3), 4.60 (s, 1H, -CH-), 6.80-7.85 (m, 8H, Ar-H); ^{13}C NMR (DMSO-*d6*): δ 21.0 (C-10), 27.4 (C-12), 55.8 (-OCH_3), 56.4 (-OCH_3), 64.0 (C-5), 114.8 (C-15 and C-17), 115.0 (C-21 and C-23), 123.0 (C-13), 126.2 (C-20 and C-24), 130.6 (C-14 and C-18), 132.4 (C-6), 136.0 (C-19), 144.4 (C-2), 152.2 (C-7), 158.8 (C-22), 160.6 (C-9), 163.2 (C-16), 196.2 (C-11); MS for $C_{22}H_{21}N_3O_3S$: 407.12 (MH^+, 100%), 408.10 (MH^{+1}, 26%), 409.08 (MH^{+2}, 5.8%); Elemental analysis: Calculated: C, 64.85; H, 5.19; N, 10.30; Found: C, 64.86; H, 5.18; N, 10.28.

2.7. 1-(5-(4-chlorophenyl)-2-(4-methoxyphenyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4f).

Obtained from 5-(4-methoxyphenyl)-1,3,4-thiadiazole-2-amine (1b, 2.07g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 84% (3.45g), m.p. 150-152^{o}C. IR (KBr, cm^{-1}): γ 2960 (C-H), 1740 (>C=O), 1624 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H, -$COCH_3$), 2.90 (s, 3H, -CH_3), 3.84 (s, 6H, -OCH_3), 4.64 (s, 1H, -CH-), 7.08-7.90 (m, 8H, Ar-H); ^{13}C NMR (DMSO-*d6*): δ 21.6 (C-10), 27.2 (C-12), 55.6 (-OCH_3), 64.8 (C-5), 114.6 (C-15 and C-17), 124.0 (C-13), 126.4 (C-20 and C-24), 128.2 (C-21 and C-23) 130.4 (C-14 and C-18), 132.2 (C-6) 132.4 (C-22), 141.2 (C-19), 144.6 (C-2), 152.2 (C-7), 160.6 (C-9), 163.0 (C-16), 196.4 (C-11); MS for $C_{21}H_{18}ClN_3O_2S$: 411.06 (MH^+, 100%), 413.08 (MH^{+1}, 37.2%),

412.08 (MH^{+2}, 25.4%); Elemental analysis: Calculated: C, 61.23; H, 4.40; Cl, 8.61; N, 10.20; Found: C, 61.22; H, 4.42; Cl, 8.62; N, 10.18.

2.8. 1-(2-(4-chlorophenyl)-7-methyl-5-phenyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4g).

Obtained from 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine (1c, 2.11g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) benzaldehyde (3a, 1.06g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 81% (3.08), m.p. 146-148ºC. IR (KBr, cm^{-1}): γ 2960 (C-H), 1726 (>C=O), 1624 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): *δ* 2.20 (s, 3H, -$COCH_3$), 2.96 (s, 3H, -CH_3), 4.68 (s, 1H, -CH-), 7.22-7.85 (m, 9H, Ar-H); ^{13}C NMR (DMSO-*d6*): *δ* 21.2 (C-10), 27.4 (C-12), 64.6 (C-5), 126.4 (C-20 and C-24), 126.8 (C-22), 128.6 (C-21 and C-23), 128.8 (C-13), 129.0 (C-15 and C-17), 129.8 (C-14 and C-18), 132.2 (C-6), 138.2 (C-16), 143.6 (C-19), 143.8 (C-2), 150.8 (C-7), 158.8 (C-9), 196.2 (C-11); MS for $C_{20}H_{16}ClN_3OS$: 381.07 (MH^+, 100%), 383.10 (MH^{+1}, 38.2), 382.08 (MH^{+2}, 24.0%), 384.08 (10.0%); Elemental analysis: Calculated: C, 62.92; H, 4.24; Cl, 9.28; N, 11.02; Found: C, 62.90; H, 4.22; Cl, 9.24; N, 11.00.

2.9. 1-(2-(4-chlorophenyl)-5-(4-methoxyphenyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4h).

Obtained from 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine (1c, 2.11g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-methoxybenzaldehyde (3b, 1.36g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 85% (3.49g), m.p. 156-158ºC. ^{1}H NMR ($CDCl_3$): *δ* 2.30 (s, 3H, -$COCH_3$), 2.98 (s, 3H, -CH_3), 3.85 (s, 3H, -OCH_3), 4.60 (s, 1H, -CH-), 6.80-8.00 (m, 8H, Ar-H); ^{13}C NMR (DMSO-*d6*): *δ* 21.6 (C-10), 27.2 (C-12), 56.2 (-OCH_3), 64.4 (C-5), 115.2 (C-21 and C-23), 125.8 (C-20 and C-24), 129.0 (C-13), 129.2 (C-15 and C-17), 129.8 (C-14 and C-18), 132.0 (C-6), 136.4 (C-19), 136.8 (C-16), 144.0 (C-2), 151.2 (C-7), 158.8 (C-22), 160.6 (C-9), 196.8 (C-11); MS for $C_{21}H_{18}ClN_3O_2S$: 411.08 (MH^+, 100%), 413.07 (MH^{+1}, 37.0%), 412.08 (MH^{+2}, 24.6%), 414.08 (9.0%); Elemental analysis: Calculated: C, 61.24; H, 4.40; Cl, 8.61; N, 10.20; Found: C, 61.26; H, 4.38; Cl, 8.58; N, 10.16.

2.10. 1-(2,5-bis(4-chlorophenyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-α]pyrimidine-6-yl)ethanone (4i).

Obtained from 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine (1c, 2.11g, 10.00mmol), acetylacetone (2.00g, 20.00mmol) 4-chlorobenzaldehyde (3c, 1.40g, 10.00mmol) and PTSA (2.50g, 15.00mmol) as yellow solid, yield 83% (3.44g), m.p. 164-166ºC. IR (KBr, cm^{-1}): γ 2956 (C-H), 1760 (>C=O), 1628 (C=N) cm^{-1}; ^{1}H NMR ($CDCl_3$): *δ* 2.32 (s, 3H, -$COCH_3$), 2.96 (s, 3H, -CH_3), 4.66 (s, 1H, -CH-), 7.20-7.80 (m, 8H, Ar-H); ^{13}C NMR (DMSO-*d6*): *δ* 21.2 (C-10), 27.0 (C-12), 64.2 (C-5), 126.4 (C-20 and C-24), 128.8 (C-21 and C-23), 129.0 (C-15 and C-17), 129.2 (C-13), 129.6 (C-14 and C-18), 132.4 (C-6), 132.8 (C-22), 137.2 (C-16), 142.0 (C-19), 143.8 (C-2), 151.8 (C-7), 160.4 (C-9), 196.4 (C-11); MS for $C_{20}H_{15}Cl_2N_3OS$: 415.02 (MH^+, 100%), 417.03 (MH^{+1}, 68.8%), 416.02 (MH^{+2}, 24.6%), 418.02 (16.4%), 419 (11.0%); Elemental analysis: Calculated: C, 57.70; H, 3.62; Cl, 17.03; N, 10.09; Found: C, 57.68; H, 3.64; Cl, 17.02; N, 11.04.

2.11. Biological assay.

2.11.1. Antimicrobial activity

The synthesized compounds 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-*α*)pyrimidine-6-yl)ethanone (4a-i) were screened for *in vitro* antibacterial activity against *Bacillus cereus* (MTCC 8372), *Staphylococcus aureus* (MTCC 96) (gram-positive bacteria) *Escherichia coli* (MTCC 724) and Klebsiella pneumonia (gram-negative bacteria) using agar disc diffusion [29] method. The compounds (4a-i) were dissolved in DMF (dimethylformamide) at concentrations 50 and 100µg/mL and placed on the inoculated plates. After allowing at 4ºC for 2h, they were incubated at 37ºC for 24h. Tetracycline was used as the standard drug, and the inhibition zone was measured in millimeters. Besides, *in vitro* antifungal screening [30] of the compounds (4a-i) was carried out against *Aspergillus flavus* (MTCC873), *Aspergillus niger* (MTCC 281), *Fusarium oxysporum* (MTCC 284), and *Fusarium moniliform* (MTCC 156) using Nystatin as standard drug. The microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of all the synthesized compounds summarized in Table 1. The compounds were stable in the Nutrient agar and Potato dextrose agar. The MIC for fungal strains was performed using a 96-well plate. The fungi were maintained on potato dextrose agar (PDA) medium at 28ºC. Six replicate determinations were performed for all the compounds. Results were taken as a mean of at least three determinations.

2.11.2. Antioxidant activity.

The scavenging effect on nitric oxide was measured according to the method of Marcocci *et al.*, [31] with a little modification [32]. Initially, 4 mL of a drug solution was added to 1 mL of sodium nitroprusside (SNP) solution (25 mM) in a test tube and incubated at 29ºC for 2 hr. 2 mL aliquot was diluted from the incubated solution with 1.2 mL Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore that was formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with *N*-1-naphthyl ethylenediamine dihydrochloride was immediately read at 550 nm. The concentration was determined from a standard curve (y = mx+c) of sodium nitrite salt-treated in the same way with Griess reagent. Inhibition of nitrite formation by the drug or the standard plant antioxidant (Ascorbic acid) was calculated relative to the control.

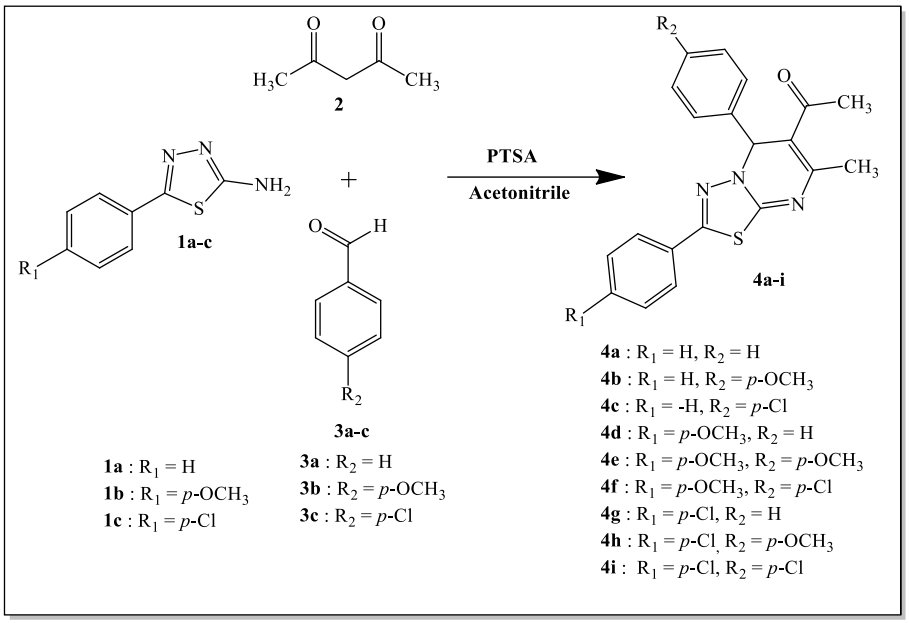
$$\text{Inhibition \%} = 100\ (A_{control} - A_{test}) / A_{control}$$

where, A_{test} is the absorbance of the control reaction mixture excluding the test compound/drug solution, and $A_{control}$ is the absorbance of the test compounds/drug solution.

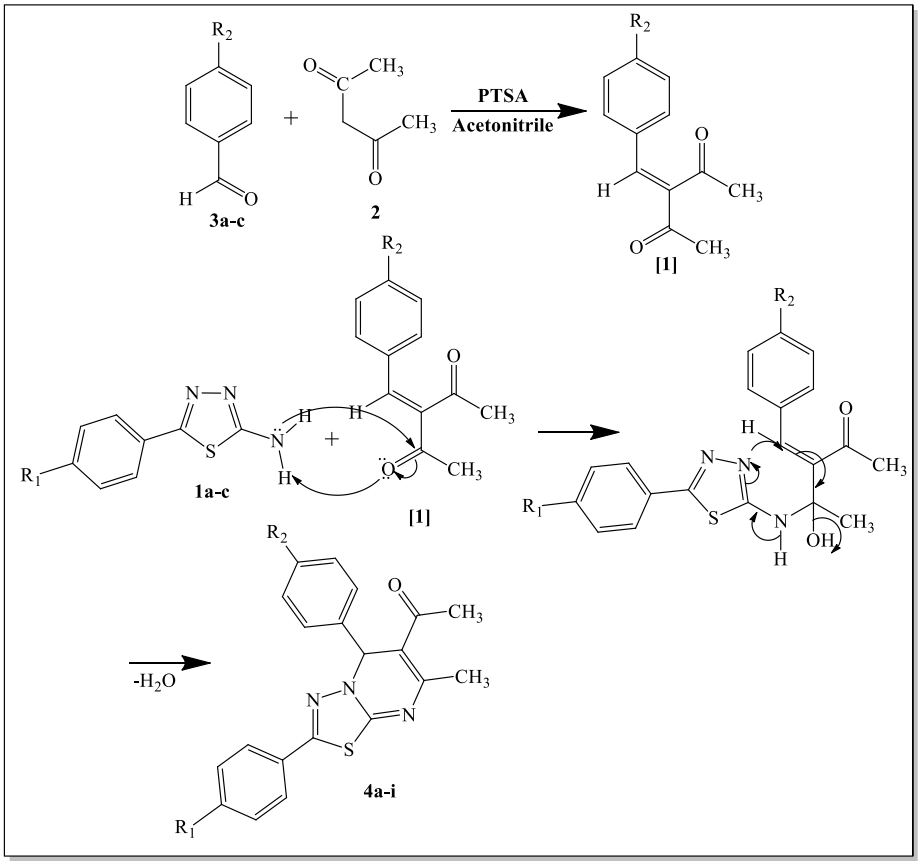
3. Results and Discussion

The synthesis of 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-*α*)pyrimidine-6-yl)ethanone (4a-i) in good yield was achieved by the cyclo-condensation reaction of active methylene acetylacetone (2) with substituted aromatic aldehydes (3a-c) and 2-amino-5-phenyl-[1,3,4]thiadiazole (1a-c) in the presence of PTSA [33] in acetonitrile as solvent (Scheme 1). The proposed mechanism for the above cyclo-condensation reaction is, as shown in Scheme 2. Initially, the *in situ* preparation of condensation intermediate (1) was achieved by the Knoevenagel condensation between active methylene compound acetylacetone with substituted aromatic aldehydes presence acidic PTSA. Meanwhile, the 2-amino-(1,3,4)-

thiadiazole reacts with condensation intermediate (1) with the elimination of water molecule to yield the target product 1-(7-methyl-2,5-diphenyl-5*H*-[1,3,4]thiadiazolo(3,2-*α*)pyrimidine-6-yl)ethanone (4a-i).



Scheme 1. Synthesis of bicyclic [1,3,4]thiadiazole[3,2-α]pyrimidine analogs.



Scheme 2. Plausible mechanism for the formation of thiadiazolyl-pyrimidine analogs.

The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass, and elemental analysis. The IR spectra of (4a-i) showed the stretching vibration bands at around 2950-2972 cm^{-1} corresponding to the presence of –CH- group, vibration bands 1610-1628 cm^{-1} for –C=H group and vibration band 1726-1760 cm^{-1} indicates the presence of >C=O

group in the compound. The ^{1}H NMR spectra of compounds (4a-i) showed the signals due to -CH_3 proton in the region δ 2.20 to 2.32 ppm, singlet peaks of –CO-CH_3 group appeared in the region δ 2.89 to 2.98 ppm, while singlet peak of –CH- group appeared in the region δ 4.60 to 4.68 ppm and all aromatic protons at δ 6.80 to 8.00 ppm. The absence of –NH_2 singlet peak in the region δ 8.40 to 9.20 ppm confirms the condensation product's formation. In ^{13}C NMR spectra presence of additional peaks in the range of δ 21.0 to 21.6 ppm (C-10), δ 27.0 to 27.4 ppm (C-12), and δ 64.0 to 264.8 ppm (C-5) was observed. All synthesized compounds 4(a-i) showed MH^+ as a base peak in the mass spectra.

3.1. Structure-activity relationship.

3.1.1. Antimicrobial assay.

The series of synthesized compounds (4a-i) contains one of the nucleosides base pyrimidine moiety. We expected good antimicrobial activity, as shown in Table-1. The results revealed that the compounds 4e showed an excellent antibacterial effect against all the tested strains. This may be due to the presence of –OCH_3 group on both para positions of the phenyl rings of thiadiazole as well as pyrimidine moieties. 4b and 4h were found to moderate, and 4d and 4f showed less activity. It may be due to the presence of –OCH_3 group on one of the phenyl rings of thiadiazole or pyrimidine ring. The compounds 4c and 4i were less active against bacterial strains, but they possess good antifungal activity. It may be the presence of –Cl group at the para position of the phenyl ring of pyrimidine, while 4e, 4g, and 4h compounds showed moderate antifungal activity and the remaining compounds showed less antifungal activity.

3.1.2. Antioxidant activity.

The antioxidant activity of the bicyclic [1,3,4]thiadiazolo[3,2-α]pyrimidine derivatives (4a-i) was evaluated *in vitro* by the nitric oxide radical scavenging assay. Most of the compounds tested significantly inhibited nitric oxide radical levels compared to that of the standard antioxidant ascorbic acid used in the study, as shown in Table-2. The compounds 4c, 4e, 4f, and 4i exhibited a strong scavenging effect on the stable nitric oxide radical, with respective to IC_{50} values of 2.50±0.65, 2.48±0.60, 2.50±0.60, and 2.50±0.67 μg mL-1. These values are slightly lower than the positive control standard antioxidant ascorbic acid, indicating that compounds with chloro and methoxy group at the pyrimidine ring's para position and found to be the most potent antioxidant agents towards nitric oxide. The remaining compounds showed moderate activity.

4. Conclusions

In conclusion, we have synthesized the novel one-pot three-component chemical transformation of bicyclic [1,3,4]thiadiazolo[3,2-α]pyrimidine (4a-i) derivatives using electron-rich substituent on the phenyl ring of the aldehydes and acetylacetone. Here there is no need for using V_2O_5/FAp catalyst preparation.

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.

Compounds	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	80	280	75	260	85	250	90	265	65	250	55	265	60	270	75	280
4b	**15**	**130**	**20**	**140**	**20**	**145**	**15**	**135**	60	290	65	285	50	280	60	275
4c	60	250	45	255	55	260	50	240	**20**	**135**	**10**	**120**	**20**	**125**	**15**	**120**
4d	**20**	**150**	**25**	**165**	**40**	**210**	**35**	**195**	60	250	50	220	65	270	55	230
4e	**10**	**130**	**15**	**145**	**10**	**120**	**10**	**135**	25	135	30	155	40	150	45	165
4f	**25**	**150**	**20**	**140**	**30**	**160**	**35**	**210**	90	285	95	275	50	280	55	260
4g	65	280	85	290	90	275	80	270	25	135	30	155	20	140	45	165
4h	**35**	**185**	**40**	**215**	**30**	**200**	**30**	**210**	35	215	40	220	45	210	30	215
4i	60	240	75	260	55	250	50	250	**10**	**120**	**15**	**125**	**20**	**130**	**15**	**130**
Tetracycline	**5**	**120**	**10**	**120**	**12**	**120**	**8**	**120**	---	---	---	---	---	---	---	---
Nystatin	---	---	---	---	---	---	---	---	**08**	**100**	**10**	**100**	**15**	**100**	**12**	**100**

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

Table 2. Antioxidant activity (IC_{50}/µg mL^{-1}) of the synthesized compounds (4a-i) against nitric oxide.

Compounds	4a	4b	**4c**	4d	**4e**	**4f**	4g	4h	**4i**	**Ascorbic acid**
Values	2.50±0.40	2.52±0.45	**2.50±0.65**	2.51±0.42	**2.48±0.60**	**2.50±0.60**	2.55±0.40	2.52±0.45	**2.50±0.67**	**5.35±0.68**

Values are the means of three replicates ± standard deviation. A lower IC_{50} value indicates better scavenging activity.

This method delivers a remarkable yield (80–86%) of the target products 80-90 minutes with low-cost PTSA compared to the V_2O_5/FAp catalyst. All the synthesized compounds have been investigated for their *in vitro* antimicrobial and antioxidant activity.

Among the synthesized compounds, 4e showed an excellent antibacterial effect, 4c and 4i possess good antifungal activity, and 4c, 4e, 4f, and 4i show excellent antioxidants in comparison with standard drugs. Hence, it could be a promising drug for microbial infections.

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Conflicts of Interest

The authors declare no conflict of interest.

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