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Novel Synthesis and Antimicrobial Activity of 7-Substituted Derivatives of 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*b*]-pyrimidine-6-carbonitrile

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ABSTRACT

2-Amino-5-phenyl-1,3,4-thiadiazole on reaction with ethyl-2-cyano-3,3-*bis*(methylthio)acrylate in the presence of *N,N'*-dimethyl formamide and catalytic amount of anhydrous potassium carbonate afforded 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*b*]pyrimidine-6-carbonitrile under similar experimental condition, compounds 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*b*]pyrimidine-6-carbonitrile on treatment independently with aryl amines/ heteryl amines/phenols containing active methylene group yielded corresponding 7-substituted derivatives. All these newly synthesized compounds were screened for antimicrobial activity.

KEYWORDS

Pyrimido-thiadiazoles, Antimicrobial activity.

INTRODUCTION

The synthesis of novel thiadiazole derivatives and investigation of their chemical and biological behaviour have gained more importance in recent decades, various substituted thiadiazole compounds are associated with diverse pharmacological activities. The specific pharmacological activities include antibacterial [1], antifungistic [2], anti-inflammatory [3], antidepressant [4], antileishmanial [5], antioxidant [6], anticonvulsant [7], antitumoural [8], antitubercular [9], carbonic anhydrase inhibitor [10] and cardio-protective action [11,12]. Recently, small heterocyclic thiadiazolidinones (TDZD) were described as the first non-ATP competitive GSK-3 β inhibitors [13] as potential drugs for the treatment of Alzheimer's disease.

The chemistry of pyrimidine and its derivatives has been studied extensively since the past century due to their diverse biological activities [14-17]. Some pyrimidine derivatives give stable and good quality nanomaterials with numerous important electrical and optical properties [18]. They contained important interesting structural coordinating substitute ligand in supra-molecular metallo-grid like architectures [19] and in novel organic/inorganic hybrid types of molecular wires [20]. In addition, pyrimidines play important role for many biochemical processes, including sucrose and cell wall polysaccharide metabolism [21] and also as in medicinal chemistry [22]. They also

Asian Journal of Organic & Medicinal Chemistry

Volume: 4

Year: 2019

Issue: 1

Month: January–March

pp: 40–45

DOI: <https://doi.org/10.14233/ajomc.2019.AJOMC-P171>

Received: 28 December 2018

Accepted: 8 March 2019

Published: 30 March 2019

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posses anticancer [23], anticonvulsant, anti HIV-1 and selective hepatitis B virus inhibiting activities [24]. Some annulated pyrimidines are used in the treatment of cardiovascular diseases [25], human cancer produced by on cogenic activation of RET [26], insomnia [27] and a sexual blood stages of all type of malaria [28].

The increasing importance of pyrimidine and its derivative as intermediate for the synthesis of useful biological compounds prompted us to develop a new method for their synthesis from ethyl-2-cyano-3,3-bis(methylthio)acrylate with 2-amino-5-phenyl[1,3,4]thiadiazole leading to the synthesis of 7-substituted derivatives of 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile and its derivatives.

EXPERIMENTAL

All the chemicals and reagents were purchased from commercial suppliers. Melting points of synthesized compounds were determined by open capillary tubes and are uncorrected. Purity of all the products was routinely checked by thin layer chromatography (TLC) on pre-coated sheets of silica gel-C plates of 0.25 mm thickness using UV Chamber for detection. Perkin-Elmer FT-IR spectra were recorded in KBr pallets on infrared spectrophotometer. Bruker advance spectrophotometer 300 MHz was used to record ^1H and ^{13}C NMR spectra in DMSO- d_6 using TMS as internal standard. Mass spectra were recorded on FT-VC-7070 H mass spectrometer using EI technique at 70 eV. All the reactions were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN-O rapid analyzer.

Synthesis of 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-b]pyrimidine-6-carbonitrile (3): A mixture of 2-amino-5-phenyl-1,3,4-thiadiazole (1) (0.01 mol) and ethyl-2-cyano-3,3-bis(methylthio)acrylate (2) (0.01 mol) was refluxed in the presence of 10-15 mL of N,N'-dimethyl formamide (DMF) and the catalytic amount of anhydrous potassium carbonate for 5 h. The reaction mixture was cooled to room temperature and poured into ice-cold water. The separated solid product was filtered, washed with water, and recrystallized from alcohol to give pure product 3 (Scheme-I).

Synthesis of 7-substituted derivatives of 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile: A mixture of compound 3 (10 mmol) was refluxed independently with different substituted aryl amines, substituted phenols, heteryl amines and compounds containing an active methylene group (10 mmol) was refluxed independently in the presence of anhydrous potassium carbonate (10 mg) in DMF (10 mL) for 3-6 h. Then reaction mixture was cooled to room temperature and poured into ice cold water, the separated crystalline solid obtained was filtered and washed with cold

water and recrystallized with ethanol to afford 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-b]pyrimidine-6-carbonitrile derivatives.

Now, 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-b]pyrimidine-6-carbonitrile (3) and different substituted aryl amines/phenols/heteryl amines and compound containing active methylene group was refluxed separately in the presence of anhydrous K_2CO_3 and DMF to afford 7-substituted derivatives of 7-(methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile (4a-f, 5a-c, 6a-d and 7a-d) (Scheme-II).

Spectral and analytical data

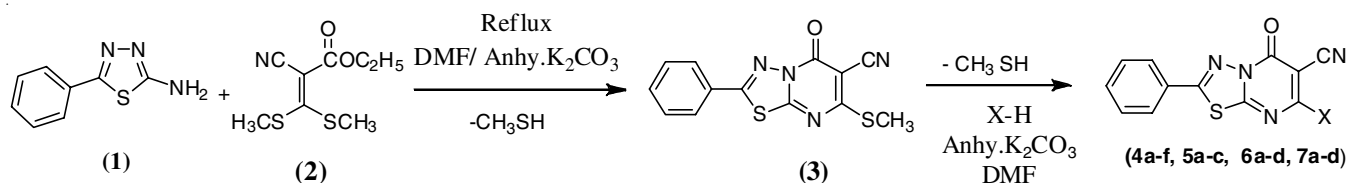
7-(Methylthio)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile (3): m.p. 203-205 °C, yield: 85 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1654 (-CO); ^1H NMR (300MHz, DMSO- d_6 , ppm): δ 2.27 (s, 3H, SCH_3), 7.55-8.09 (m, 5H, Ar-H); EI-MS (m/z : RA %): 301 ($\text{M}^{+} + 1$, 100 %); ^{13}C NMR (300 MHz, DMSO- d_6 , ppm): δ 12.81, 12.81 (- SCH_3), 115.00 (-CN), 126.49, 127.53, 128.91, 129.46, 129.75, 131.32, 194.19. Elemental analysis of $\text{C}_{13}\text{H}_4\text{N}_4\text{OS}_2$ calcd. (found) %: C, 51.98 (51.95); H, 2.68 (2.65); N, 18.65 (18.63).

7-(4-Fluorophenylamino)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile (4a): m.p. 150-152 °C, yield 65 %. IR (KBr, ν_{max} , cm^{-1}): 3290 (-NH), 2210 (-CN), 1654 (-CO); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ 4.30 (br, s, 1H, -NH), 7.72-8.10 (m, 10H, Ar-H); EI-MS (m/z : RA %): 364 ($\text{M}^{+} + 1$, 100 %); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 72.02, 116.02, 120.40, 129.02, 130.41, 134.22, 142.10, 159.02, 164.36, 172.10. Elemental analysis of $\text{C}_{18}\text{H}_{10}\text{N}_5\text{OSF}$ calcd. (found) %: C, 59.50 (59.48); H, 4.48 (4.46); N, 20.76 (20.75).

7-(4-Chlorophenylamino)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile (4b): m.p. 215-217 °C, yield 67 %. IR (KBr, ν_{max} , cm^{-1}): 3240 (-NH), 1660 (-CO), 2215 (-CN); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ 4.05 (br, s, 1H, -NH), 6.90-7.20 (m, 5H, Ar-H), 7.40-8.60 (m, 4H, Ar-H); EI-MS (m/z : RA %): 379 ($\text{M}^{+} + 1$, 100 %), 381 ($\text{M}^{+} + 3$, 33 %). Elemental analysis of $\text{C}_{18}\text{H}_{10}\text{N}_5\text{OSCl}$ calcd. (found) %: C, 56.92 (56.90); H, 2.65 (2.63); N, 18.44 (18.42).

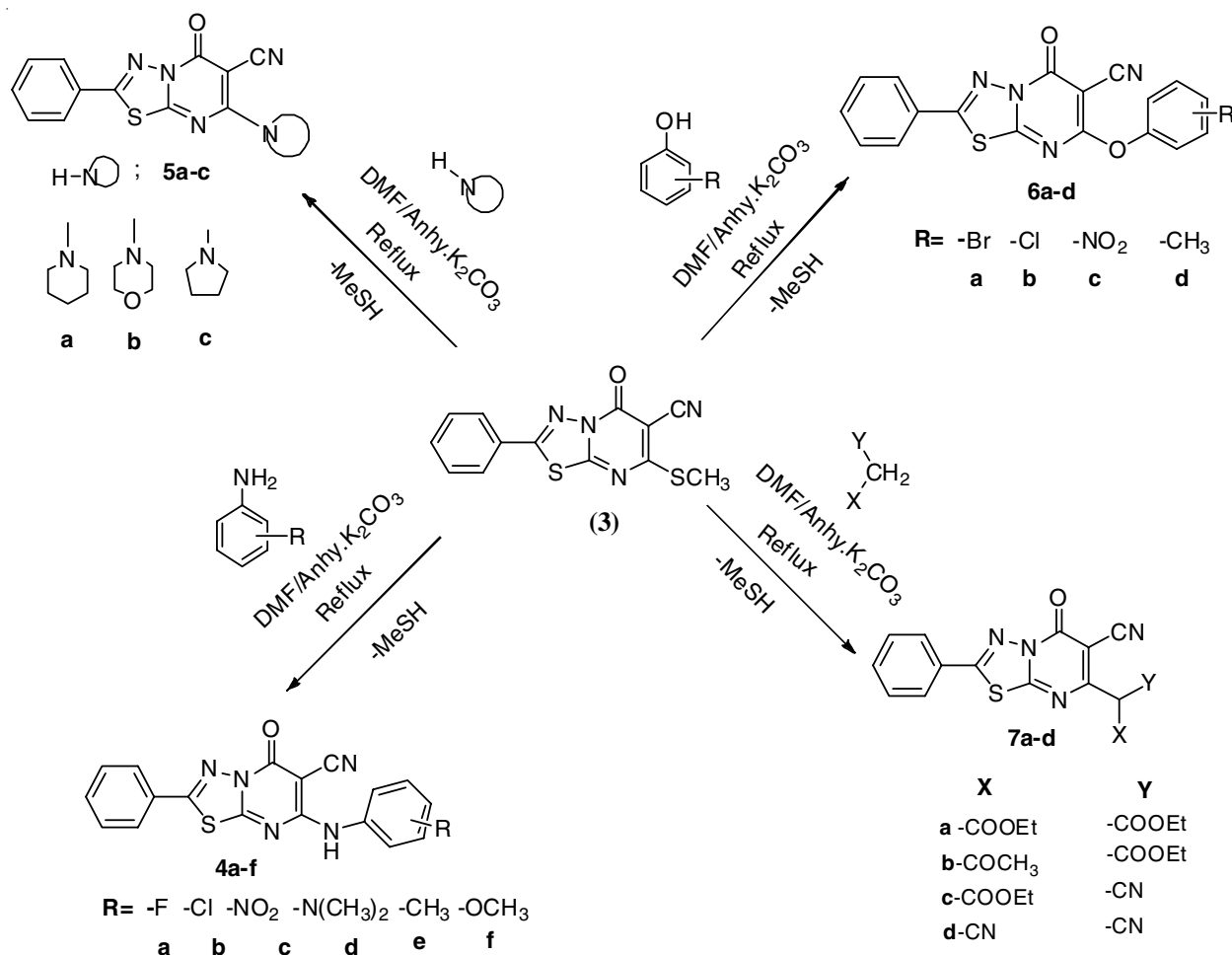
7-(4-Nitrophenylamino)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile (4c): m.p. 193-195 °C, yield 61 %. IR (KBr, ν_{max} , cm^{-1}): 3230 (-NH), 2220 (-CN), 1675 (-CO); ^1H NMR (400 MHz, DMSO- d_6 , ppm): δ 4.10 (br, s, 1H, -NH), 7.06-7.20 (m, 5H, Ar-H), 7.28-8.69 (m, 4H, Ar-H); EI-MS (m/z : RA %): 390 ($\text{M}^{+} + 1$, 100 %). Elemental analysis of $\text{C}_{18}\text{H}_{10}\text{N}_6\text{O}_3\text{S}$ calcd. (found) %: C, 55.38 (55.35); H, 2.58 (2.56); N, 21.53 (21.51).

7-(4-(Dimethylamino)phenylamino)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carbonitrile



X- different substituted Aromatic amines, heteryl amines, phenol and active methylene compounds

Scheme-I



Scheme-II

(4d): m.p. 219-221 °C, yield 72 %. IR (KBr, ν_{max} , cm^{-1}): 3250 (-NH), 2240 (-CN), 1665 (-CO); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 3.06 (s, 6H, -N(CH₃)₂), 4.12 (br, s, 1H, -NH), 6.38 - 7.22 (m, 5H, Ar-H), 7.26-8.05 (m, 4H, Ar-H); EI-MS (*m/z*: RA %): 388 ($M^{+} + 1$, 100 %). Elemental analysis of C₂₀H₁₆N₆O₂S calcd. (found) %: C, 55.38 (55.35); H, 2.58 (2.56); N, 21.53 (21.50).

7-(4-Methylphenylamino)-5-oxo-2-phenyl-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (4e): m.p. 214-216 °C, yield 69 %. IR (KBr, ν_{max} , cm^{-1}): 3255 (-NH), 1690 (-CO), 2222 (-CN); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 2.40 (s, 3H, -CH₃), 4.20 (br, s, 1H, -NH), 6.50-7.15 (m, 5H, Ar-H), 7.25-8.10 (m, 4H, Ar-H); EI-MS (*m/z*: RA %): 379 ($M^{+} + 1$, 100 %). Elemental analysis of C₁₉H₁₃N₅O₂S calcd. (found) %: C, 59.49 (59.47); H, 3.65 (3.62); N, 19.49 (19.45).

7-(4-Methoxyaminophenylamino)-5-oxo-2-phenyl-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (4f): m.p. 235-237 °C, yield 74 %. IR (KBr, ν_{max} , cm^{-1}): 3268 (-NH), 1682 (-CO), 2215 (-CN); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 3.80 (s, 3H, -OCH₃), 4.05 (br, s, 1H, -NH), 6.70-7.20 (m, 5H, Ar-H), 7.30-8.02 (m, 4H, Ar-H); EI-MS (*m/z*: RA %): 375 ($M^{+} + 1$, 100 %). Elemental analysis of C₁₉H₁₃N₅O₂S calcd. (found) %: C, 60.79 (60.76); H, 3.49 (3.45); N, 18.66 (18.65).

7-Piperidino-5-oxo-2-phenyl-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (5a): m.p. 140-142 °C, yield 58 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1639 (-CO); ¹H NMR

(400 MHz, DMSO-*d*₆, ppm): δ 2.53-2.59 (t, 4H, -2CH₂), 2.72-2.88 (m, 6H, -3CH₂), 7.56-8.11 (m, 5H, Ar-H); EI-MS (*m/z*: RA %): 340 ($M^{+} + 1$, 100 %). Elemental analysis of C₁₇H₁₅N₅O₂S calcd. (found) %: C, 60.52 (60.50); H, 4.48 (4.45); N, 20.76 (20.74).

7-Morpholino-5-oxo-2-phenyl-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (5b): m.p. 159-161 °C, yield 60 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1639 (-CO); ¹H NMR (400 MHz, DMSO-*d*₆, ppm): δ 2.53-2.59 (t, 4H, -2CH₂), 2.72-2.88 (m, 4H, -OCH₂), 7.06-7.98 (m, 5H, Ar-H); EI-MS (*m/z*: RA %): 338 ($M^{+} + 1$, 100 %). Elemental analysis of C₁₆H₁₃N₅O₂S calcd. (found) %: C, 56.63 (56.60); H, 3.86 (3.85); N, 20.64 (20.62).

7-(Pyrrolidino)-5-oxo-2-phenyl-7-(pyrrolidino)-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (5c): m.p. 177-179 °C, yield 71 %. IR (KBr, ν_{max} , cm^{-1}): 2120 (-CN), 1645 (-CO); ¹H NMR (200 MHz, DMSO-*d*₆, ppm): δ 2.1 (t, 4H, -2CH₂), 2.95 (t, 4H, -NCH₂), 7.4-7.9 (m, 5H, Ar-H); EI-MS (*m/z*: RA %): 323 ($M^{+} + 1$, 100 %). Elemental analysis of C₁₆H₁₃N₅O₂S calcd. (found) %: C, 59.43 (59.40); H, 4.38 (4.36); N, 26.07 (26.05).

7-(4-Fluorophenoxy)-5-oxo-2-phenyl-5H-[1,3,4]-thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (6a): m.p. 150-152 °C, yield 65 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1654 (-CO); ¹H NMR (200 MHz, DMSO-*d*₆, ppm): δ 4.12 (s, 1H, N-H), 7.22-8.10 (m, 9H, Ar-H); EI-MS (*m/z*: RA %): 364 ($M^{+} + 1$, 100 %);

^{13}C NMR (300 MHz, DMSO- d_6 , ppm): δ 72.40, 116.05, 120.15, 130.10, 134.70, 144.12, 150.14, 157.02, 160.10, 174.40. Elemental analysis of $\text{C}_{18}\text{H}_{10}\text{N}_5\text{OSF}$ calcd. (found) %: C, 59.50 (59.48); H, 4.48 (4.45); N, 20.76 (20.74).

7-(4-Chlorophenoxy)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (6b): m.p. 230-232 °C, yield 67 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1650 (-CO); ^1H NMR (200 MHz, DMSO- d_6 , ppm): δ 7.20-7.70 (m, 5H, Ar-H), 7.80-8.30 (m, 4H, Ar-H); EI-MS (m/z : RA %): 380 ($\text{M}^{++} + 1$, 100%), 382 ($\text{M}^{++} + 3$, 33 %). Elemental analysis of $\text{C}_{18}\text{H}_9\text{N}_4\text{O}_2\text{S}$ calcd. (found) %: C, 56.77 (56.75); H, 2.36 (2.35); N, 14.71 (14.70).

7-(4-Nitrophenoxy)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (6c): m.p. 220-222 °C, yield 65 %. IR (KBr, ν_{max} , cm^{-1}): 2205 (-CN), 1670 (-CO); ^1H NMR (200 MHz, DMSO- d_6 , ppm): δ 7.4-8.2 (m, 9H, Ar-H); EI-MS (m/z : RA %): 391 ($\text{M}^{++} + 1$, 100 %). Elemental analysis calculated data for $\text{C}_{18}\text{H}_9\text{N}_5\text{O}_4\text{S}$ calcd. (found) %: C, 55.24 (55.22); H, 2.32 (2.30); N, 17.89 (17.85).

7-(*p*-Tolyloxy)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (6d): m.p. 218-220 °C, yield 70 %. IR (KBr, ν_{max} , cm^{-1}): 2225 (-CN), 1650 (-CO); ^1H NMR (200 MHz, DMSO- d_6 , ppm): δ 2.1 (s, 3H, Ar- CH_3), 7.10-8.02 (m, 9H, Ar-H); EI-MS (m/z : RA %): 360 ($\text{M}^{++} + 1$, 100 %). Elemental analysis calculated data for $\text{C}_{19}\text{H}_{12}\text{N}_4\text{O}_2\text{S}$ calcd. (found) %: C, 63.32 (63.30); H, 3.36 (3.35); N, 19.49 (19.47).

Diethyl-2-(6-cyano-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-7-yl)malonate (7a): m.p. 185-187 °C, yield 74 %. IR (KBr, ν_{max} , cm^{-1}): 2210 (-CN), 1666 (-CO); ^1H NMR (300 MHz, DMSO- d_6 , ppm): δ 2.53-2.63 (t, 6H, 2 CH_3), 2.88 (q, 4H, 2 CH_2), 4.60 (s, 1H, -CH), 7.33-8.11 (m, 5H, Ar-H); EI-MS (m/z : RA %): 412 ($\text{M}^{++} + 1$, 100 %); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 12.75, 14.47, 34.18, 115.58, 126.34, 126.47, 128.85, 129.23, 129.47, 131.36, 175. Elemental analysis for $\text{C}_{19}\text{H}_{16}\text{N}_4\text{O}_5\text{S}$ calcd. (found) %: C, 55.33 (55.30); H, 3.91 (3.91); N, 13.58 (13.56).

Ethyl 2-(6-cyano-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-7-yl)-3-oxobutanoate (7b): m.p. 190-192 °C, Yield 69 %. IR (KBr, ν_{max} , cm^{-1}): 2222 (-CN), 1750 (-COOC $_2\text{H}_5$), 1640 (-CO); ^1H NMR (300 MHz, DMSO- d_6 , ppm): δ 1.6 (t, 3H, - CH_3), 2.2 (s, 3H, COCH $_3$), 3.4 (q, 2H, -OCH $_2$), 4.25 (s, 1H, -CH), 7.20-7.96 (m, 5H, Ar-H); EI-MS (m/z : RA %): 382 ($\text{M}^{++} + 1$, 100 %); Elemental analysis of $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$ calcd. (found) %: C, 55.54 (55.51); H, 3.69 (3.66); N, 19.17 (19.16).

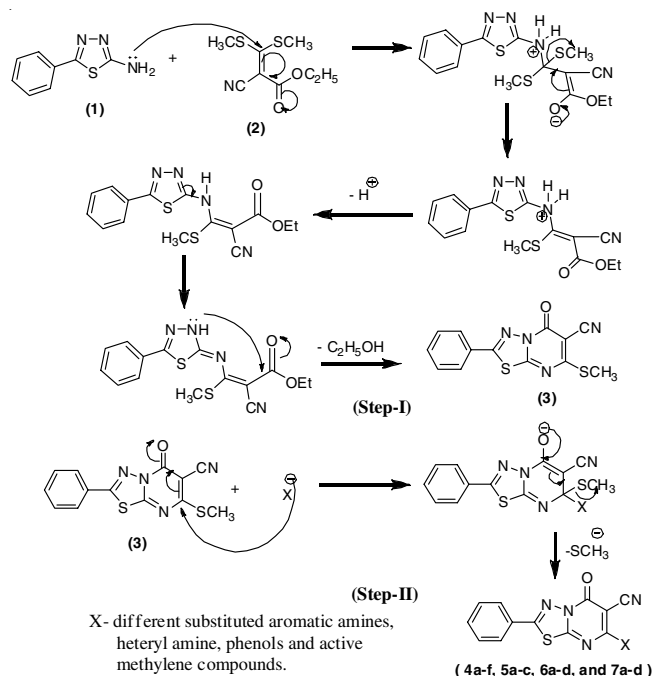
Ethyl 2-cyano-2-(6-cyano-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-7-yl)acetate (7c): m.p. 169-171 °C, yield 76 %. IR (KBr, ν_{max} , cm^{-1}): 2230 (-CN), 1740 (-COOC $_2\text{H}_5$); ^1H NMR (300 MHz, DMSO- d_6 , ppm): δ 2.6 (t, 3H, - CH_3), 3.6 (q, 2H, -OCH $_2$), 4.0 (s, 1H, -CH), 7.02-7.90 (m, 5H, Ar-H); EI-MS (m/z : RA %): 365 ($\text{M}^{++} + 1$, 100 %); Elemental analysis of $\text{C}_{17}\text{H}_{11}\text{N}_5\text{O}_3\text{S}$ calcd. (found) %: C, 55.88 (55.86); H, 3.03 (3.00); N, 19.17 (19.15).

7-(Dicyanomethyl)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (7d): m.p. 180-182 °C, yield 73 %. IR (KBr, ν_{max} , cm^{-1}): 2235 (-CN), 1660 (-CO); ^1H NMR (300 MHz, DMSO- d_6 , ppm): δ 4.20 (s, 1H, -CH), 7.40-7.80 (m, 5H, Ar-H); EI-MS (m/z : RA %): 318 ($\text{M}^{++} + 1$, 100 %); Elemental analysis of $\text{C}_{15}\text{H}_6\text{N}_6\text{OS}$ calcd. (found) %: C, 56.60 (56.58); H, 1.90 (1.88); N, 26.40 (26.37).

RESULTS AND DISCUSSION

In present work, we have reported one pot synthesis of 3-cyano-2-methylthio-4-oxo-7-phenyl pyrimido[2, 3-*d*]-1,3,4-thiadiazole (**3**) and their 2-substituted derivatives from the reaction of ethyl-2-cyano-3,3-bis(methylthio)acrylate (**2**) with 2-amino-5-phenyl-1,3,4-thiadiazole in the presence of DMF and catalytic amount of anhydrous K_2CO_3 .

The structure of compound **3** was assigned on the basis of elemental analysis, IR, ^1H NMR, ^{13}C NMR and mass spectral data. A mechanism for the formation of compound **3** and its derivatives can be adducted as shown in **Scheme-III**. Compound **3** possess a replaceable active methyl-thio group at 7-position which is activated by the ring 1-nitrogen atom and the electron withdrawing cyano group at 6-position. Hence, it is deduced that compound **3** would become principal precursor for the synthesis of its 7-substituted derivatives. Appropriately, 7-substituted derivatives of compound **3** have been prepared by reacting compound **3** with selected N-, O- and C-nucleophiles like aryl amines, heteryl amines, substituted phenols and compounds containing active methylene group. These reactions resulted in the formation of 7-substituted derivatives of compound **3**. According to this method, compound **3** on reaction independently with 4-fluoro/chloro/nitro/dimethylamino/methyl and 4-methoxy aniline in presence of DMF and catalytic amount of anhydrous K_2CO_3 to afforded 7-(4-fluorophenylamino, 4-chlorophenylamino, 4-nitrophenylamino, 4-*N,N*-dimethylphenylamino, 4-methylphenylamino, 4-methoxyphenylamino)-5-oxo-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6-carbonitrile (**4a-f**), respectively.



Under similar experimental conditions compound **3** reacted independently with heterylamines like piperidine, morpholine, and pyrrolidine to obtain 5-oxo-2-phenyl-7-(piperidino, morpholino and pyrrolidino)-5H-[1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6-carbonitrile (**5a-c**), respectively.

Compound **3** was again refluxed in K_2CO_3 and DMF independently with different substituted phenols like *p*-bromo, *p*-chloro, *p*-nitro and *p*-methyl phenol to afford 7-(4-bromophenoxy, 4-chlorophenoxy, 4-nitrophenoxy and 4-methyl phenoxy)-5-oxo-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (**6a-d**). Under similar experimental conditions, compound **3** was reacted independently with diethyl malonate, ethyl acetoacetate, ethyl cyanoacetate and malanonitrile to obtain 7-(diethyl malonate, ethyl acetoacetate, malanonitrile and ethyl cyanoacetate)-5-oxo-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile derivatives (**7a-d**).

All the synthesized compounds (**4a-f**, **5a-c**, **6a-d** and **7a-d**) exhibited absorption bands in their IR spectra in the range of 2250-2105 and 1710-1640 cm^{-1} due to -CN stretching and -CO stretching, respectively. 1H NMR, ^{13}C NMR and mass spectral data are also in agreement with the structure assigned to the synthesized compounds (**4a-f**, **5a-c**, **6a-d** and **7a-d**).

Antimicrobial activity: The synthesized compounds (**4a-f**, **5a-c**, **6a-d** and **7a-d**) of the series 7-(methylthio)-5-oxo-2-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6-carbonitrile (**3**) and its 7-substituted derivatives were evaluated for their antibacterial activity against Gram positive species *S. aureus* and Gram negative species *E. coli* and *P. vulgaris* by paper diffusion method. All the synthesized compounds were dissolved in dimethyl sulphoxide (DMSO). The synthesized compounds exhibited zone of inhibition at 10-18 mm in diameter where as standard Penicillin exhibited zone of inhibition at 10 and 15 mm in diameter against *S. aureus* and 10 and 18 mm in diameter against *E. coli* and 10 and 14 mm in diameter against *P. vulgaris* and standard Streptomycin exhibited 15, 18 and 14 mm zone of inhibition against *S. aureus*, *E. coli* and *P. vulgaris*, respectively (Table-1). Nystatin exhibited zone of inhibition at 5 and 18 mm in diameter against *A. fumigates*. Amongst these synthesized compounds (**4b**, **5a** and **7c**) shows higher zone of

inhibition against *S. aureus*. Compounds (**4c**, **5b** and **7a**) shows higher zone of inhibition against *E. coli* and compounds (**4b**, **5a** and **7a**) shows higher zone of inhibition against *P. vulgaris* as compared to other compounds. The compounds (**3** and **7d**) shows higher zone of inhibition against *A. fumigates* as compared to other compounds (Table-1).

Conclusion

In summary, a new series of 7-substituted pyrimido[2,3-*d*]-thiadiazole derivatives using an efficient, green and easy protocol were synthesized and characterized. The pyrimido[2,3-*d*]-thiadiazole derivatives and its 7-substituted derivatives were evaluated for antimicrobial activity. The synthesized compounds can be easily isolated by simple workup technique, less expensive, short time, requires ambient reaction condition, and give excellent yields.

ACKNOWLEDGEMENTS

The authors are grateful to The Principal, Yeshwant Mahavidyalaya, Nanded, India for providing research facilities and UGC, New Delhi, India (File no. 41-230/2012) (SR) for financial support. One of the authors (JAA) also thanks UGC, New Delhi for the award of Teacher fellowship under Faculty Improvement Programme (File no. 37-03/2012, WRO). The Director, CSIR-IICT Hyderabad and Punjab University, Chandigarh, India for spectral analysis.

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Entry	Compd.	Zone of inhibition in mm* (activity index) ^{std}			
		<i>S. aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>A. fumigatus</i>
1	3	13	11	13	35
2	4a	10	11	11	13
3	4b	15	14	21	13
4	4c	ND	18	13	13
5	4d	ND	10	ND	12
6	4e	11	18	13	ND
7	4f	10	14	12	ND
8	5a	15	14	21	13
9	5b	ND	18	13	13
10	5c	10	11	12	05
11	6a	ND	14	12	13
12	6b	10	12	10	ND
13	6c	10	ND	ND	05
14	6d	12	10	11	ND
15	7a	ND	16	14	ND
16	7b	10	12	10	ND
17	7c	13	12	12	13
18	7d	11	18	13	18
19	Penicillin	26	40	18	-
20	Streptomycin	40	35	34	-
21	Nystatin	-	-	-	40

ND= Not detected

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