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A one pot, efficient and eco-friendly synthesis of 1,3,4-thiadiazolo[3,2-a] pyrimidine scaffold *via* Aza–Michael addition and intramolecular cyclo-elimination reactions in poly ethylene glycol (PEG)

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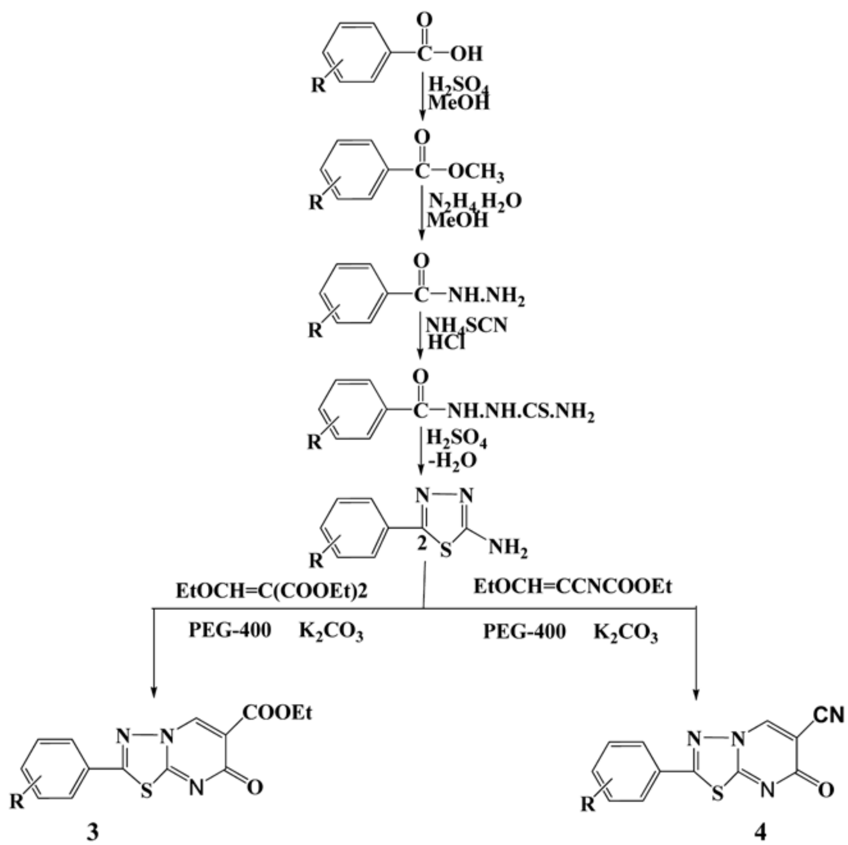
Potassium carbonate in poly (ethylene glycol-400) has been found to be a highly effective and efficient medium for the straight forward, convenient, one pot and green synthesis of ethyl 2-substituted phenyl-7-oxo-7*H*-[1,3,4]- thiadiazolo [3,2-a]- pyrimidine-6-corboxylate and -6-corbonitrile through intramolecular cyclo-elimination of Michael adducts formed between the reaction of 2-amino-5 substituted thiadiazoles with diethyl-2- (ethoxymethylene) malonate and ethyl-2- cyano-3-ethoxyacrylate respectively. The structures of all the new compounds have been elucidated using IR, ^{1}H and ^{13}C NMR, mass spectral data and elemental analyses.

Keywords: Substituted phenyl, 1,3,4-thiadiazolo, pyrimidine, carboxylate, carbonitrile, antimicrobial activity, fungicidal activity

Michael addition of nucleophiles to electron deficient alkenes is one of the most powerful and widely used synthetic tools for the formation of carbon-carbon and carbon-hetero bonds in organic chemistry[1-4]. Hetero Michael additions, *viz*. aza-Michael, thia-Michael, *etc*. are the most exploited organic reactions and are the mainstay of efficient synthetic tools for the construction of druggable heterocyclic scaffolds and natural products[5-7]. Construction of molecular architecture by two or more bond formation in one-step operation *via* Michael reaction has been one of the current interest in synthetic organic chemistry[8, 9]. Although reports on double Michael additions with cyclic and acyclic acrylates and enones are numerous, but with acrylates having electron withdrawing group at α-carbon along with an ethoxy group at β-carbon with heterocyclic amines are scare. The continual upsurge in facile, convenient and nonpolluting synthetic procedure urges chemists to increase tools of their arsenal. The growing awareness of the pressing need for greener and more sustainable technologies has focus attention on the use of alternative reaction media that circumvent the problems associated with traditional volatile organic solvents. One such approach to address this challenge is the elimination or reduction of the threat of use of volatile organic solvents to achieve the most important goal of green chemistry. Poly (ethylene glycol), a biologically acceptable polymer used in drug delivery has been emerged as an alternative and interesting green reaction media in organic synthesis. It has replaced many other neoteric solvents such as ionic liquid, super-carbon dioxide and micellar systems whose toxicological properties and biodegradability have not been established completely. Its unique properties such as thermal stability, cost effectiveness, commercial availability, non-volatility, reduced toxicity, ease of recyclability, non-halogenated nature and high polarity for solubilization with wide variety of organic solvents render PEG a designer solvent in organic synthesis. Although Michael addition reactions in various solvents have been accomplished but only few reports in PEG are currently known[10-12]. There have been reports on Michael addition reactions, catalysed by an in-expensive commercial compound, K_2CO_3 in various solvents[13-16], but in PEG are scare[10].

The high therapeutic properties of the compounds incorporating nitrogen heterocycles have encouraged the medicinal chemists to synthesize large number of novel therapeutic agents. Thiadiazole and pyrimidine rings containing only nitrogen atom, are the active cores of various bio-active molecules. Therefore, the heterocyclic system resulting from the annulations of pyrimidine ring with bio-active thiadiazole is an attractive scaffold. 1,3,4-Thiadiazoles are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities including antibacterial[17-21] antifungal[22-25], antitubercular[26,27], analgesic[28], and leishmaniadal[29] agents. Pyrimidines represent one of the most active classes of compounds, possessing a wide spectrum of biological activity[30]. Pyrimidines and their fused ring derivatives have a broad spectrum of biological activity, best known as heterocylic core of the nucleic acid bases. These ring systems are often incorporated into drugs designed for analgesic and anti-inflammatory[30] ,anticancer [31], antiviral [32]and antihypertensive [33]activites. The cyano group is a stable and useful functional group that can be transformed to various other functional groups such as acyl, carboxy, formyl, carbamoyl, *etc*[31-34]. The past seven decades has witnessed the transition of organic nitriles from a position of laboratory curiosities to that of large tonnage chemicals of commercial importance. On the other hand, reactions involving C-C bond formation are one of the mainstays in synthetic organic chemistry. The use of nitrile for C-C bond formation reactions occupies an important position in organic chemistry[35-37].

In the light of the above literature facts and abundance we report herein, an eco-benign and one-pot aza-Michael annulations of a functionalized pyrimidine on thiadiazole and using potassium carbonate an effective and efficient catalyst (Scheme I). A plausible mechanism for the formation of titled 1,3,4- thiadiazole – [3,2-a] pyrimidine scaffold is given in Scheme II. The nitrogen atom at position -3 *i.e*. -NH (sp^3) of 1,3,4- thiadiazole is more nucleophilic than = NH (sp^2). Therefore, the Michael addition is initiated through –NH and not with =NH. The titled compounds by virtue of having functionalized pyrimidine on thiadiazole in a single molecule may show pronounced biocidal activity. The

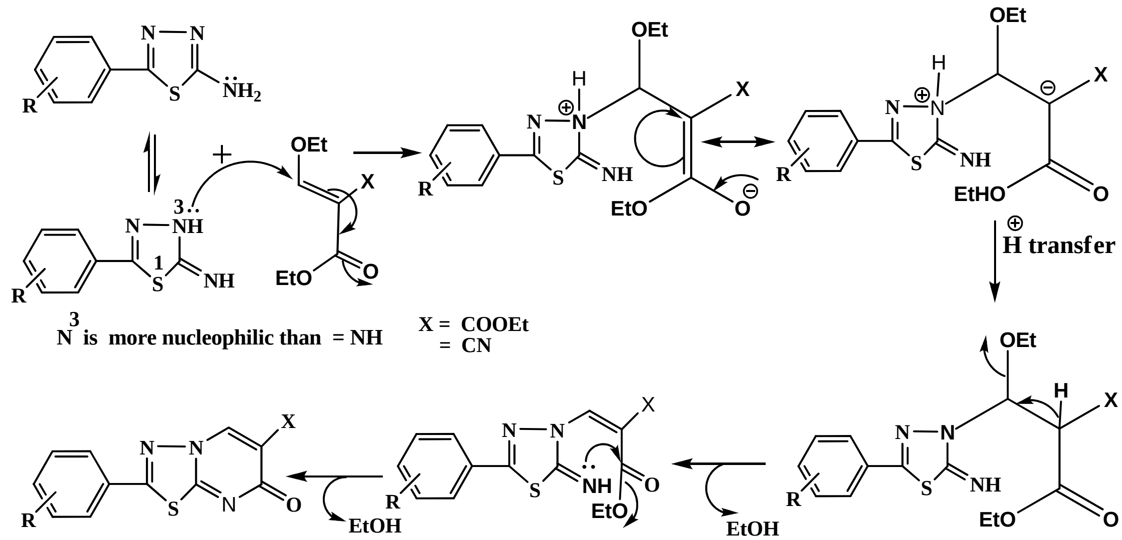


3
a: R=4-Cl
b: R=4-CH$_3$
c: R=2,4-Cl$_2$

4
a: R=2-Cl
b: R=2,4-Cl$_2$
c: R=4-NO$_2$

d: R=4-NO$_2$

Scheme — I



Scheme II

structures of these compounds was established by the IR, ^{1}H NMR, ^{13}C NMR and elemental analysis.

The required starting material 2-amino-5-substituted aryl-1,3,4 thiadiazoles was prepared by following known method[38,21]. The appropriate thiosemicarbazide was treated with Conc. H_2SO_4 dropwise. The resultant paste-like mass so obtained was cooled and poured in to cold water. In neutralisation with ammonia, solid product was obtained, which was filtered, washed and recrystallised from aqueous ethanol to furnish the corresponding thzadiazole derivatives.

Antimicrobial Activity

The antimicrobial activity of synthesized compounds **3a-d** and **4a-c** was determined *in vitro* against four bacterial strains. For this study, the test cultures of bacterial strains *Escherichia coli*, *Salmonella typhii*, *Bacillus subtilis* and *Staphylococcus aureus* were maintained in nutrient agar slants at 37ºC. The antimicrobial activity of compounds against test bacteria was determined by agar well diffusion method[39,40] using standard antibiotic ciprofloxacin as positive control and DMSO as negative control. All the experiments were performed in triplicate.

The results of present investigation showed that compounds **3a, 3b, 3d, 4c** have promising activity against all the test organisms. Except **3c** all the compounds showed moderate to good activity against *staphylococcus aureus.* Most of the other compounds were either active or inactive against test organisms. Compounds **3a, 3b** and **4c** is found to be most effective against all test organisms (Table I).

Table I — Zone of inhibition in mm at concentration 100 ug/mL

Compd	*B. subtilis*	*S. aureus*	*E.coli*	*S. typhi*
3a	32	23	28	27
3b	24	19	23	23
3c	-	12	-	-
3d	21	20	11	-
4a	10	12	12	-
4b	10	17	11	-
4c	25	21	22	19
Ciprofloxacin	35	46	40	39

Fungicidal Activity

In vitro antifungal activity of all compounds were studied against two fungal strains, *Candida albicans* and *Aspergillus niger*. Itraconazole was employed as standard to compare the results. Among all the compounds, compound **3d** and **4c** has good antifungal activity. Compounds **3a, 3b** and **4a** displayed moderate antifungal activity and the remaining compounds are found to be inactive (Table II).

Experimental Section

All the melting points were determined on a Cintex melting point apparatus and are uncorrected. All reagents were purchased commercially and used without further purification. IR spectra were recorded using KBr pellets on a Perkin-Elmer

Table II — Antifungal activity of compounds **3a-b** and **4a-c**

Compd	Fungal species and MIC (Ug/M2)	
	C. albicans	*A. niger*
3a	5	7
3b	8	10
3c	0	0
3d	17	19
4a	8	10
4b	0	0
4c	16	18
Itraconazole	24	26
Control	-	-

BX series FT-IR spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Varian Gemini 300 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer at 75.5 MHz. Chemical shifts are reported in δ (ppm) using TMS as internal standard. Mass spectral measurements were carried out at 70 eV by EI method on a Jeol JMC-300 spectrometer. The homogeneity of the compounds was checked by TLC (silica gel, hexane/ethyl acetate).

General procedure for synthesis of ethyl 2-(4-substituted phenyl)-7-oxo-7*H*-[1,3,4] – thiadiazolo – [3, 2-a]-pyrimidine-6-carboxylates, 3a-d

To a mixture of diethyl ethoxyethylene malonate (2.0 mmol) and K_2CO_3 (0.15 mmol) in polyethylene glycol (5 mL) was added 2-amino-5-substituted phenyl-1,3,4-thiadiazole (2.0 mmol) and reaction mixture was allowed stir at 60ºC for 2-3 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with water and neutralized with 1N HCl. The precipitate thus formed was filtered to give the product. The crude products **3a-d** was purified by column chromatography and characterized by ^{1}H and ^{13}C NMR, mass spectral data, and elemental analysis.

Ethyl 2-(4-chlorophenyl)-7-oxo-7*H*-[1,3,4]-thiadiazolo- [3,2-a]-pyrimidine-6-carboxylate, 3a: Colourless solid. Yield 70%. m.p.190ºC. IR: 3050 (C-H arom), 1710 (C=O), 1675 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 7.75-7.58 (dd, 4H, arom), 6.7 (s,1H, pyrimidine ring proton), 4.20 (q, J = 7Hz, 2H,OCH_2), 1.25 (t, J = 7Hz,3H, CH_3); ^{13}C NMR: δ 14.2, 61.3, 113.1,128.5, 128.9, 129.6, 136.7, 143.3, 156.0, 163.0, 163.7, 168; MS: *m/z* (M^+) 335 (100%).

Ethyl-7-oxo-2-*p*-tolyl-7*H*-[1,3,4]-thiadiazolo-[3,2-a] -pyrimidine-6-carboxylate, 3b: Brown solid. Yield 65%. m.p.205ºC. IR: 3020 (C-H arom), 1705 (C=O), 1680 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 7.64-7.25 (dd, 4H, arom), 6.7 (s,1H, pyrimidine ring proton), 4.20 (q, J = 7Hz, 2H,OCH_2), 1.25 (t, J = 7Hz,3H, CH_3); ^{13}C NMR: δ 14.2, 21.3, 61.6, 113.2,127.5, 129.6, 143.3, 156.0, 156.5, 163.1, 163.7, 168.0; MS: *m/z* (M^+) 315 (100%).

Ethyl-2-(2,4-chlorophenyl)-7-oxo-7*H*-[1,3,4]-thiadia zolo-[3,2-a]-pyrimidine-6-carboxylate, 3C: Light yellow solid. Yield 72%. m.p.180ºC. IR: 3055 (C-H arom), 1722 (C=O), 1690 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 7.68-7.35 (m, 3H, arom), 6.7 (s,1H, pyrimidine ring proton), 4.20 (q, J = 7Hz, 2H,OCH_2), 1.25 (t, J = 7Hz,3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 14.2, 61.3, 113.1, 127.2, 128.7, 129.6, 132.2 135.7, 143.5, 156.1, 163.0, 163.7, 168; MS: *m/z* (M^+) 368.97 (100%).

Ethyl 2-(4-nitrophenyl)-7-oxo-7*H*-[1,3,4]-thiadia zolo-[3,2-a]-pyrimidine-6-carboxylate, 3d: Yellow solid. Yield 67%. m.p.186ºC. IR: 3050 (C-H arom), 1710 (C=O), 1685 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 8.30-8.06 (m, 4H, arom), 6.7 (s,1H, pyrimidine ring proton), 4.20 (q, J = 7Hz, 2H,OCH_2), 1.27 (t, J = 7Hz,3H, CH_3); ^{13}C NMR: δ 14.2, 61.4, 113.1, 124.2, 130.4, 136.7, 143.5, 150.1, 156.5, 163.0, 163.7, 168.5; MS: *m/z* (M^+) 346.04 (100%).

General procedure for synthesis of 2-(substituted phenyl)-7-oxo-7*H*-[1,3,4]-thiadiazolo-[3,2-a]-pyrimidine-6-carbonitriles, 4a-C

To a mixure of ethyl-2-cyano-3-ethoxy acrylate (2.0 mmol) and K_2CO_3 (0.15 mmol) in polyethylene glycol (5 mL) was added 2-amino-5-substituted phenyl-1,3,4 – thiadiazole (2.0 mmol) and reaction mixture was allowed stir at 60°C. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with water and neutralized with 1N HCl. The precipitate thus formed was filtered to give the product. The crude products **4a-c** were purified by coloumn chromatography and characterized by ^{1}H and ^{13}C NMR, mass spectral data, and elemental analysis.

2-(2-Chlorophenyl)-7-oxo-7*H*-[1,3,4]-thiadiazolo- [3, 2-a]-pyrimidine-6-carbonitrile, 4a: Light brown solid. Yield 60%. m.p.156ºC. IR: 3015 (C-H arom), 2205 (C≡N streching), 1682 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 7.70-7.45 (m, 4H, arom), 6.5 (s,1H, pyrimidine ring proton); ^{13}C NMR: δ 99.6, 114.0, 126.8, 130.3, 130.9, 131.5, 132.4, 133.8, 143.8, 159.6, 163.4, 168.3; MS: *m/z* (M^+) 287.99 (100%).

2-(2,4-Dichlorophenyl)-7-oxo-7*H*-[1,3,4]-thiadiazolo [3,2-a]pyrimidine-6-carbonitrile, 4b: Brown solid. Yield 65%. m.p.174ºC. IR: 3005 (C-H arom), 2219 (C≡N streching), 1693 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 7.68-7.47 (m, 3H, arom), 6.5 (s,1H, pyrimidine ring proton); ^{13}C NMR: δ 99.7, 114.1, 127.3, 128.1, 129.1, 129.9, 132.4, 135.8, 143.6, 159.6, 163.0, 168.1; MS: *m/z* (M^+) 321.98 (100%).

2-(4-Nitrophenyl)-7-oxo-7*H*-[1,3,4]-thiadiazolo-[3,2 -a]-pyrimidine-6-carbonitrile, 4c: Yellow solid. Yield 64%. m.p.160ºC. IR: 3030 (C-H arom), 2210 (C≡N streching), 1695 cm^{-1} (C=O of pyrimidine ring); ^{1}H NMR (DMSO-d_6): δ 8.30-8.06 (m, 4H, arom), 6.5(s,1H, pyrimidine ring proton); ^{13}C NMR: δ 99.6, 114.3, 124.3, 130.3, 136.9, 143.3, 150.0, 159.6, 163.4, 168.3; MS: *m/z* (M^+) 299.01 (100%).

Conclusion

In the present investigation, a series of new thiadiazolo pyrimidines have been synthesized in a single step by cycloelimination of the adduct formed by double aza- Michael addition. The synthesized compounds have been screened for their antifungal and antibacterial activity. The activity reveals that the synthesized compounds possess moderate to good activity profile. The insights gained from this study will be useful for development of new anti-infective agents. These reaction provides a high selective access to reaction sequences leading to the molecular motifs while combining structural, diversity, versatility and eco-compatibility.

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