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Dihetero double Michael addition in PEG-400: Synthesis of 2,3-dihydro- [2,3-c]-[1,2,4]-triazole scaffold

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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 diethyl/ethyl cyano-5-[substituted phenyl-2,3-dihydro-[1,3,4]-thiadiazolidino - [2, 3-c] – [1, 2, 4]-triazol -2- yl] – malonate/acetate through intramolecular cyclo- elimination of Michael adducts formed between the reaction of 4-amino-5- substituted phenyl-3-mercapto-1,2,4-triazole **1** 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-thiadiazolidine, 1,2,4-triazole, malonate, acetate, 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 up surge 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 neotric 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.

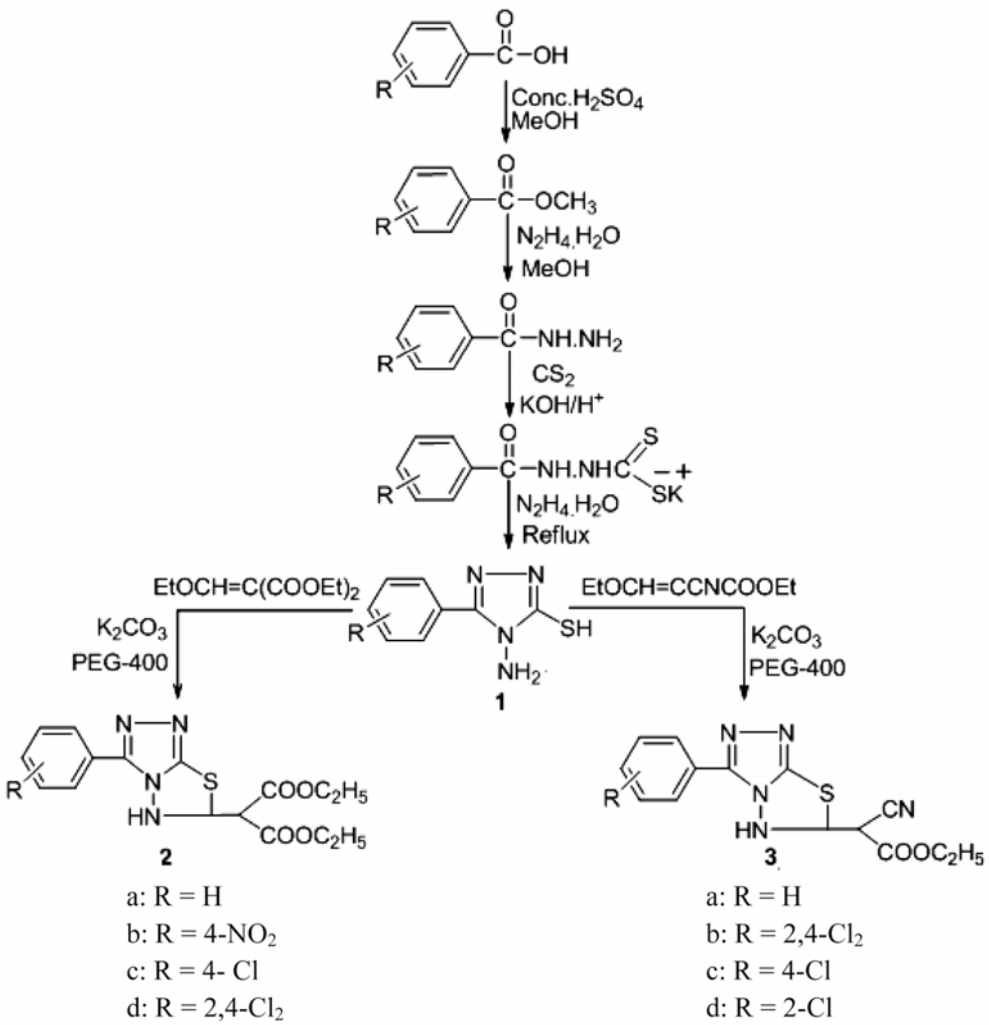
Thiadiazoles are known to have wide spectrum of biological activities such as anti-bacterial[17], pesticidal[18-20], anti-fungal[17] anti-tubercular[21], carbonic

anhydrase inhibitors[22], anti-inflammatory[23], analgesic[23], anti-depressant[24-26], anti-HIV and anti-cancer activities.

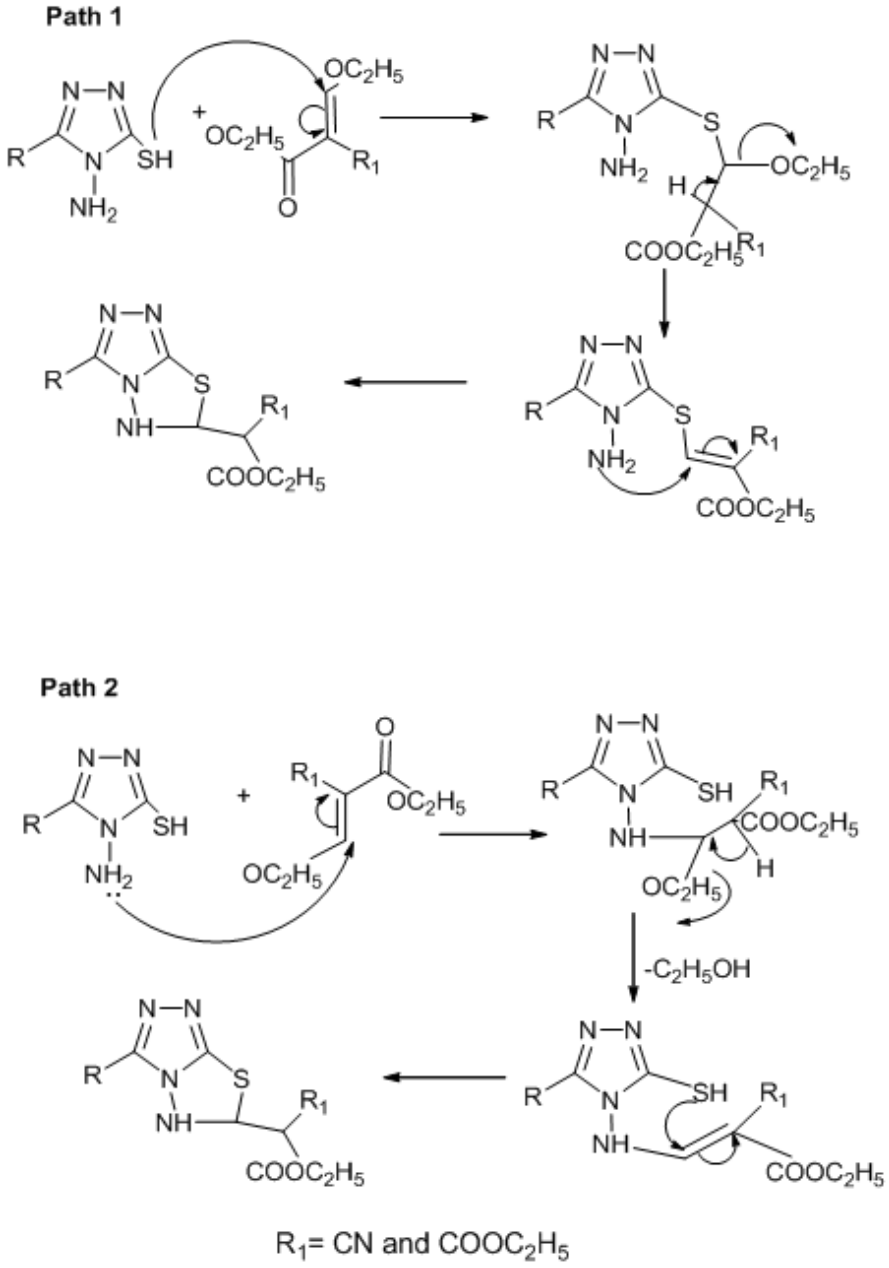
Triazoles have received considerable importance because of their wide biological applications. They exhibit antifungal[27], anti-inflammatory[28], anti-cancer[29] and anti-convulsant[30] activities. These are less toxic than compounds like keto-conazole and miconazole due to their selective toxicity towards fungal target enzymes. Fluconazole is one of the potent triazole containing antifungal agent.

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] 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[32-34].

In the light of the above literature facts and abundance we report herein, thiadiazole and triazole derivatives through double Michael addition using potassium carbonate an effective and efficient catalyst (Scheme I). A plausible mechanism for the formation of titled 2, 3-dihydro-[2, 3-c] – [1, 2, 4]- triazole scaffold is given in Scheme II. The titled compounds by virtue of having thiadiazole and triazole moiety in a single molecule may show pronounced biocidal



Scheme I



Scheme-II

activity. The structure of these compounds established by the IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The required starting material 4-amino-5-substituted phenyl-3-mercapto-1, 2, 4-triazole **1** was prepared according to the reported methods[20].

Antimicrobial activity

The antimicrobial of synthesized compounds **2a-d** and **3a-d** 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 37°C. The antimicrobial activity of compounds against test bacteria was determined by agar well diffusion method[35,36] using standard antibiotic ciprofloxacin as positive control and DMSO as negative control. All the experiments were performed in triplicate.

The result of present investigation showed that compounds **2b, 2c** and **3c** have promising activity against all the test organisms.

Except **2a** all the compounds showed moderate to good activity against *Staphylococcus aures*. Most of the other compounds were either weakly active or inactive against test organisms. Compounds **2b, 2c** and **3c** is found to be most effective against all test organisms (Table I).

Fungicidal screening

The fungicidal activity was evaluated against *Cephalosporium saccharii* and *Helminthosporium oryzae* by the usual agar-plate technique[37] in Czapek's a gas medium of 1000 ppm, 100 ppm, 10 ppm concentrations using Mancozeb M-45, a commercial fungicide, as standard. The compounds were tested either as solution or suspension in acetone-water 20:80 (v/v) mixture. The standard solution or suspensions of different concentration of each compound viz 10000 ppm, 1000 ppm and 100 ppm were prepared in acetone-water 20:80 (v/v) mixture. 1 ml of each concentration of the tested compound was added separately to presterilized petri dishes containing 9 ml of sterilized Czapek's agar medium to maintain the final concentrations of 1000 ppm, 100 ppm and 10 ppm. The compound was thoroughly mixed with the medium by rotating the plates on table top, thus swirling the contents. A fungal disk of 5 mm diameter cut out with the help of sterilized cork borer from the periphery of one weak old culture of test fungus already planted on the Czapek's agar medium, was inoculated in the centre of each petri-dishes containing 9 ml of Czapek's agar medium. The numbers of replications in each case were three. After 96 hr the diameter of fungal growth zone was measured. The results were expressed in terms of the percentage growth inhibition, by comparing with growth on control. Thus

$$\text{Percentage inhibition} = \frac{(C-T)\times 100}{C}$$

Where, C= diameter (in mm) of the fungal colony in control plate, T= diameter (in mm) of the fungal colony in treated plate.

The antifungal data of compounds are listed in Table II.

Among all the compounds, compound **2b** and **3c** has good antifungal activity and the remaining compounds are displayed moderate antifungal activity.

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

Compd	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhii*
2a	–	12	–	–
2b	31	24	28	27
2c	24	22	21	22
2d	weak	15	weak	–
3a	–	12	–	–
3b	10	12	–	–
3c	23	19	21	21
3d	–	–	weak	–
Ciprofloxacin	35	46	40	40

Table II — Fungicidal activity of compounds **2a-d** and **3a-d**

Compd	*Cephalosporium saccharii*			*Heliminthosporium oryzae*		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
2a	84	62	45	80	60	52
2b	100	90	75	97	68	47
2c	84	59	33	83	71	50
2d	79	45	26	83	61	42
3a	83	63	44	79	61	51
3b	78	41	26	85	60	44
3c	100	91	76	95	69	48
3d	84	58	32	83	71	50
Mancozeb M-45	100	72	58	100	86	58

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 BX series FT-IR spectrophotometer. The ^{1}HNMR spectra were recorded in CD Cl_3/DMSO-d_6 on a varian Gemini 300 MHz spectrometer. The ^{13}CNMR spectra were recorded in CD Cl_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 70ev by El 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 diethyl-5-[substituted phenyl- 2, 3-dihydro-[1, 3, 4]-thiadiazolidino-[2, 3-c]-[1, 2, 4]-triazol-2-yl]-malonate (2a-d)

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

Diethyl-5-[phenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[2,3-c]-[1,2,4]-triazol-2-yl]-malonate 2a: Colourless solid yield 68% m.p. 180**°C;** IR: 3208 (N-H), 3030 (C-H arom), 2914 (C-H aliph) 1702 (C=O), 1664 (C=N), 1592,1491 (phenyl ring), 1429 (C=C) cm-1; ^{1}H NMR (DMSO-d_6): δ , 7.34-6.95 (m, 5H, arom), 4.95 (t,1H,methyne proton of thiadiazole ring), 4.19-4.27 (q, 2H,OCH_2), 3.22 (d,1H,methylene proton), 2.21(d,1H, NH proton of thiadiazole ring), 1.43 (t,3H,CH_3); ^{13}C NMR (δ ppm) : δ 14.1, 60.6, 66.2, 124.7, 125.0, 125.7, 126.9, 129.2, 130.5, 156.5, 166.5, 190.4. MS (m/z) (M^+) 360.07 (100%).

Diethyl-5-(3-[4-nitrophenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[3,2,c]-[1,2,4]-triazol-2yl]-malonate 2b: Yellow solid yield 74% m.p. 172°C IR: 3040 (C-H arom), 1710 (C=O of pyrimidine ring), 1605 (C=N), 1525 (phenyl ring stretching) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.95-7.50 (m, 4H, arom), 4.75 (t,1H, CH proton), 4.13 (q, 2H, OCH_2 proton), 3.66 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.3, 52.6, 124.3, 127.3, 137.5, 148.7, 150.7, 166.6, 167.7, 168.6. MS (m/z) (M^+) 405.07 (100%).

Diethyl-5-[4-chlorophenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[3,2,c]-[1,2,4]-triazol-2yl]-malonate 2c: Colourless solid yield 76% m.p. 155°C IR: 3020 (C-H arom), 1715 (C=O of pyrimidine ring), 1600 (C=N), 1530 (phenyl ring) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.80-7.45 (m, 4H, arom), 4.75 (t,1H, CH proton), 4.18 (q, 2H, OCH_2 proton), 3.64 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.6, 52.6, 61.8, 128.3, 128.9, 129.5, 135.1, 151.4, 167.6, 169.8, MS (m/z) (M^+) 396.05 (100%).

Diethyl-5-[2,4-dichlorophenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[3,2,c]-[1,2,4]-triazol-2yl]-malonate 2d: Colourless solid yield 65% m.p. 165°C IR: 3030 (C-H arom), 1725 (C=O of pyrimidine ring), 1610 (C=N), 1525 (phenyl ring) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.77-7.46 (m, 3H, arom), 4.43(t,1H, CH proton), 4.14 (q, 2H, OCH_2 proton), 3.60 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.3, 52.9, 61.3, 127.7, 130.2, 130.9, 133.4, 135.9, 136.8, 151.4, 167.3, 167.9, 168.2. MS (m/z) (M^+) 429.01 (100%).

General procedure for synthesis of ethyl cyano-5-[substituted phenyl- 2, 3-dihydro-[1, 3, 4]-thiadiazolidino-[3, 2-c]-[1, 2, 4]-triazol-2-yl]-acetate, 3a-d

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

Ethyl cyano-5-[phenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[3,2-c]-1,2,4-triazol-2-yl]-acetate 3a: Colourless solid yield 60% m.p. 145°C IR: 3181 (N-H), 3096 (C-H arom), 2910 (C-H aliph), 2215

(C≡N), 1688 (C=O), 1605 (C=N), 1405 (C=C) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 8.31-7.45 (m, 5H, arom), 4.96 (t,1H,methyne proton of thiadiazole ring), 4.24 (q, 2H,OCH_2), 3.56 (d,1H, methylene proton), 2.50 (d,1H, NH proton of thiadiazole ring), 1.20(t,3H,CH_3-proton); ^{13}C NMR (δ ppm) : δ 14.6, 48.8, 55.0, 99.3, 113.7, 130.4, 126.8, 127.8, 149.6, 158.5, 168.0, 182.3. MS (m/z) (M^+)349.02(100 %).

Ethyl 2-cyano-5-[2,4-dichlorophenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-

[3,2-c]-1,2,4-triazol-2-yl]-acetate 3b: Yellow solid yield 72% m.p. 164°C IR: 3035 (C-H arom), 2210 (C≡N) 1725 (C=O of pyrimidine ring), 1615 (C=N) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.85-7.43 (m, 3H, arom), 4.80 (t,1H, CH proton), 4.20 (q, 2H, OCH_2 proton), 3.61 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.2, 64.4, 115.9, 127.7, 130.2, 130.9, 133.4, 135.9, 136.8, 151.4, 167.3, 167.9, 168.2. MS (m/z) (M^+) 382.99 (100%).

Ethyl 2-cyano-5-[4-chlorophenyl-2,3-dihydro-[1,3, 4]-thiadiazolidino-[3,2-

c]-1,2,4-triazol-2-yl]-acetate 3c: Colourless solid yield 72% m.p. 182°C IR: 3005 (C-H arom), 2210 (C≡N), 1702 (C=O of pyrimidine ring), 1605 (C=N) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.90-7.45 (m, 4H, arom), 4.45 (t,1H, CH proton), 4.18 (q, 2H, OCH_2 proton), 3.74 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.2, 37.8, 64.5, 115.6, 128.4, 128.9, 129.5, 134.6, 151.4, 163.6, 167.6, 168.3, MS (m/z) (M^+) 349.02 (100%).

Ethyl 2-cyano-5-[2-chlorophenyl-2,3-dihydro-[1,3, 4]-thiadiazolidino-[3,2-

c]-1,2,4-triazol-2-yl]-acetate 3d: Colourless solid yield 65% m.p. 152°C IR: 3010 (C-H arom), 2210 (C≡N), 1702 (C=O of pyrimidine ring), 1605 (C=N) cm-^{1}; ^{1}H NMR (DMSO-d_6): δ 7.70-7.30 (m, 4H, arom), 4.45 (t,1H, CH proton), 4.18 (q, 2H, OCH_2 proton), 3.74 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH_3 proton); ^{13}C NMR (δ ppm) : δ 14.2, 37.8, 64.5, 115.6, 127.3, 128.8, 129.5, 130.1, 132.6, 138.5, 151.4, 163.6, 167.6, 168.3, MS (m/z) (M^+) 349.02 (100%).

Conclusion

In the present investigation, a series of new heterocyles have been synthesized and screened for their antifungal and antimicrobial activity. The activity results reveal that the synthesized compounds possess moderate to good activity profiles. The insights gained in this study will be useful for development of newer anti-infective agents.

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