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

Shailendra Tiwari*a, Akeel Ahmadb & Vinod Kumar Singhc

*a Department of Chemistry, University of Allahabad, Allahabad 211 002, India
*b Department of Chemistry, DDU Gorakhpur University, Gorakhpur 273 009, India
*c Shivpati PG College Shohratgarh, Siddhart Nagar 272 205, India

E-mail: drshailendratiwariau@gmail.com

Received 19 May 2020; accepted (revised) 4 September 2020

Potassium carbonate in poly (ethylene glycol-400) has been found to be a highly effective and efficient medium for the straightforward, 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 with diethyl-2-(ethoxymethylene) malonate and ethyl -2-cyano-3-ethoxyacrylate respectively. The structures of all the new compounds have been elucidated using IR, 1H and 13C 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 chemistry1-4. 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 products5.7. 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 chemistry8,9. 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 known10-12. There have been reports on Michael addition reactions, catalysed by an inexpensive commercial compound, K2CO3 in various solvents13-16, but in PEG are scarce10. 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-bacterial17, pesticidal18-20, anti-fungal17 anti-tubercular21, carbonic
Triazoles have received considerable importance because of their wide biological applications. They exhibit antifungal, anti-inflammatory, anti-cancer and anti-convulsant 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. 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.

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 thia diazole and triazole moiety in a single molecule may show pronounced biocidal

Scheme I
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 aureus*. 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 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

\[
\text{Percentage inhibition} = \left( \frac{C-T}{C} \right) \times 100
\]

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

<table>
<thead>
<tr>
<th>Compd</th>
<th><em>Bacillus subtilis</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Salmonella typhii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>–</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2b</td>
<td>31</td>
<td>24</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2c</td>
<td>24</td>
<td>22</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>2d</td>
<td>weak</td>
<td>15</td>
<td>weak</td>
<td>–</td>
</tr>
<tr>
<td>3a</td>
<td>–</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3b</td>
<td>10</td>
<td>12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3c</td>
<td>23</td>
<td>19</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>3d</td>
<td>–</td>
<td>–</td>
<td>weak</td>
<td>–</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>35</td>
<td>46</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compd</th>
<th><em>Cephalosporium saccharii</em></th>
<th><em>Helminthosporium oryzae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 ppm</td>
<td>10 ppm</td>
</tr>
<tr>
<td>2a</td>
<td>84</td>
<td>62</td>
</tr>
<tr>
<td>2b</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>2c</td>
<td>84</td>
<td>59</td>
</tr>
<tr>
<td>2d</td>
<td>79</td>
<td>45</td>
</tr>
<tr>
<td>3a</td>
<td>83</td>
<td>63</td>
</tr>
<tr>
<td>3b</td>
<td>78</td>
<td>41</td>
</tr>
<tr>
<td>3c</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>3d</td>
<td>84</td>
<td>58</td>
</tr>
<tr>
<td>Mancozeb M-45</td>
<td>100</td>
<td>72</td>
</tr>
</tbody>
</table>
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 $^1$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 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$_2$CO$_3$ (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 (C=O stretching), 1664 (C=N), 1525 (phenyl ring) cm$^{-1}$; $^1$H NMR (DMSO-d$_6$): δ 7.95-7.60 (m, 4H, arom), 4.75 (t, 1H, CH proton), 4.14 (q, 2H, OCH$_2$ proton), 3.60 (d, 1H, CH proton), 2.21 (d, 1H, NH proton of thiadiazole ring), 1.43 (t, 3H, CH$_3$); $^{13}$C NMR (δ ppm) : δ 14.3, 52.9, 61.3, 127.7, 137.5, 148.7, 150.7, 166.6, 167.7, 168.6. MS (m/z) (M$^+$) 360.07 (100%).

Diethyl-5-[4-nitrophenyl-2,3-dihydro-[1,3,4]-thiadiazolidino-[3,2-c]-[1,2,4]-triazol-2-yl]-malonate 2b

Yellow solid yield 74% m.p. 172°C IR: 3208 (C=O stretching), 1605 (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$^+$) 429.01 (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=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=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, 132.0, 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$_2$CO$_3$ (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 (3a-d) was 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=O), 1665 (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, 61.8, 128.3, 128.9, 129.5, 135.1, 151.4, 167.6, 169.8. MS (m/z) (M$^+$) 396.05 (100%).
(C≡N), 1688 (C=O), 1605 (C=N ), 1405 (C=C) cm⁻¹; ¹H NMR (DMSO-d₆): δ 8.31–7.45 (m, 5H, arom), 4.96 (t, 1H, methyne proton of thiadiazole ring), 4.24 (q, 2H, OCH₂), 3.56 (d, 1H, methylene proton), 2.50 (d, 1H, NH proton of thiadiazole ring), 1.20 (t, 3H, CH₃ proton); ¹³C 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; ¹H NMR (DMSO-d₆): δ 7.85–7.43 (m, 5H, arom), 4.18 (q, 2H, OCH₂ proton), 3.74 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH₃ proton); ¹³C NMR (δ ppm): δ 14.2, 37.8, 64.5, 115.6, 128.4, 128.9, 129.5, 130.1, 132.6, 138.5, 151.4, 163.6, 167.6, 168.3, 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. 164°C IR: 3035 (C-H arom), 2210 (C≡N), 1725 (C=O of pyrimidine ring), 1615 (C=N) cm⁻¹; ¹H NMR (DMSO-d₆): δ 7.85–7.43 (m, 5H, arom), 4.18 (q, 2H, OCH₂ proton), 3.74 (d, 1H, CH proton), 2.0 (d, 1H, NH proton), 1.29 (t, 3H, CH₃ proton); ¹³C NMR (δ ppm): δ 14.2, 37.8, 64.5, 115.6, 128.4, 128.9, 129.5, 132.6, 138.5, 151.4, 163.6, 167.6, 168.3, MS (m/z) (M⁺) 382.99 (100%).

Acknowledgements
The authors are thankful to the Heads, Chemistry Department, DDU Gorakhpur University and University of Allahabad for departmental facilities and CDRI, Lucknow for spectral and elemental analysis. One of the authors (A. Ahamd) is thankful to University Grants Commission, New Delhi for financial assistantship.

References