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# Green synthesis of tetrahydropyrimidine analogues and evaluation of their antimicrobial activity

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ABSTRACT

It is the first report of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives, catalyzed by $ZrOCl_2$. The mild reaction conditions, excellent yields in shorter reaction time and evasion of cumbersome workup procedures make this process economically lucrative for industrial application. The novelty and highlight of the present method is the promising antibacterial and antifungal activity shown by compounds **(4a**, **4b**, **4e, 4f, 4h** and **4l)** compared to standard.



Pyrimidines and their analogues are considered as important bioactive heterocycles. Tetrahydropyrimidine is an important heterocyclic ring which is widely explored in the past decade for exhibiting interesting biological activities. In particular they are screened for antiviral, anti-inflammatory and muscarinic agonist activities.[1–3] Tetrahydropyrimidine is also responsible for salt and heat sensitivity of protein–DNA interactions.[4] However, literature reveals very few synthetic methods for synthesis of polysubstituted 1,2,3,6-tetrahydropyrimidines, which were also associated with drawbacks like low yields, high temperature, long reaction time and complicated procedures.[5] Thus, one of the major challenge before synthetic chemist is the establishment of practical synthetic method for the synthesis of tetrahydropyrimidine analogues. These challenges can mainly be overcome by the employment of multicomponent reactions in synthesis, which would give higher yields of complex product in shorter reaction time.[6]

In continuation of our work,[7] on the development of synthetic methodologies and screening of the analogues, we have discovered efficient method for the synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidine analogues. The 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidine analogues were synthesized efficiently through the three component reactions of substituted amines, substituted but-2-ynedioate and formaldehyde using $ZrOCl_2$ in water (Scheme 1). Initially, for the model reaction (**4a**), aniline (2 mmol) was treated with diethyl acetylene dicarboxylate (1 mmol) and formaldehyde (4 mmol) without use of catalyst and by using different catalysts like hydrochloric acid, sulphuric acid, nitric acid, acetic acid, sulphamic acid, sulphanilic acid and Zirconyl oxychloride ($ZrOCl_2$) at reflux temperature in water (Table 1).The $ZrOCl_2$ (Table 1, entry 8) was found to be superior catalyst for this reaction by considering the reaction time (20 min) and yield (90%) whereas the rest of the catalysts required longer reaction time (45–140 min) and yielded (57–78%) less product. In addition the $ZrOCl_2$ is easily available having good activity at low cost and is easy to handle due to its low toxicity when compared to the other used catalyst (Table 1).

To evaluate the exact concentration of $ZrOCl_2$ required for the reaction, we investigated the model reaction **(4a)** using different concentrations (Table 2) such as 2, 4, 6, 8, 10, 12 mol % the product was formed in 55, 67, 72, 85, 90, 90% respectively. The result revealed that, when the reaction was carried out in presence of 2, 4, 6 mol % of catalyst, it gave lower yield of product even after prolonged duration. While 8, 10 and 12 mol % of catalyst gave excellent yields of product in shorter time. The optimal results were obtained by 10 mol % of catalyst and this concentration was ideal to carry out reaction smoothly (Table 2, entry 5). In order to evaluate the effect of solvent, reactions were carried out in various solvent as enlisted in (Table 3). Acetonitrile and isopropyl alcohol afforded moderate yield 72% and 65%, respectively. Methanol, ethanol and aqueous ethanol (50%) resulted in good yields of 82%, 90% and 90%, respectively. Whereas, water furnished the product in excellent 90% yield (Table 3, entry 6), making it the most favorable solvent.

The possible mechanism of the reaction is shown in (Scheme 2) wherein, the reactions involving substituted amines, formaldehyde, dimethyl and diethyl acetylene dicarboxylates (substituted but-2-ynedioate) occur in the presence of 10 mol % $ZrOCl_2$ in aqueous medium. In the first step the amine reacts with the

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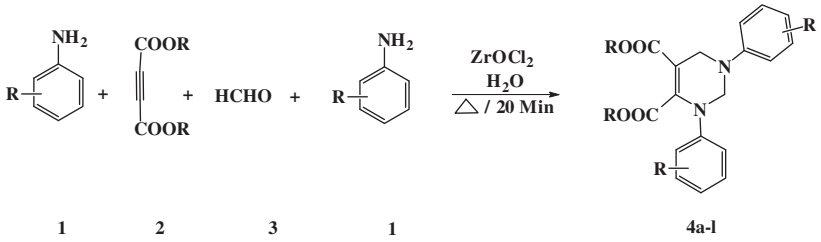



**Scheme 1.** Synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines catalyzed by $ZrOCl_2$ in water.

**Table 1**
The effect of catalyst on model reaction (**4a**)[a]

| Entry | Catalyst | Time (min) | Yield[b] (%) |
|---|---|---|---|
| 1 | No catalyst | 180 | 32 |
| 2 | Hydrochloric acid | 140 | 57 |
| 3 | Sulphuric acid | 125 | 62 |
| 4 | Nitric acid | 90 | 65 |
| 5 | Acetic acid | 75 | 72 |
| 6 | Sulphamic Acid | 55 | 76 |
| 7 | Sulphanilic Acid | 45 | 78 |
| 8 | Zirconyl oxychloride | 20 | 90 |

[a] Reaction condition: Aniline (2 mmol), diethyl acetylene dicarboxylate (1 mmol), formaldehyde (4 mmol) in water (5 mL) at reflux temperature using various catalyst (10 mol %).
[b] Isolated yields.

**Table 2**
Effect of concentration of $ZrOCl_2$ on model reaction (**4a**)[a]

| Entry | Catalyst (Mol %) | Time (min) | Yield[b] (%) |
|---|---|---|---|
| 1 | 2 | 60 | 55 |
| 2 | 4 | 45 | 67 |
| 3 | 6 | 25 | 72 |
| 4 | 8 | 25 | 85 |
| 5 | 10 | 10 | 90 |
| 6 | 12 | 20 | 90 |

[a] Reaction condition: Aniline (2 mmol), diethyl acetylene dicarboxylate (1 mmol), Formaldehyde (4 mmol) in water (5 mL) at reflux temperature using various concentration of $ZrOCl_2$.
[b] Isolated yields.

**Table 3**
Screening of solvent for model reaction (**4a**)[a]

| Entry | Solvent | Time (min) | Yield[b] (%) |
|---|---|---|---|
| 1 | Acetonitrile | 25 | 72 |
| 2 | Isopropyl alcohol | 30 | 65 |
| 3 | Methanol | 15 | 82 |
| 4 | Ethanol | 13 | 90 |
| 5 | Ethanol–water (50%) | 10 | 90 |
| 6 | Water | 10 | 90 |

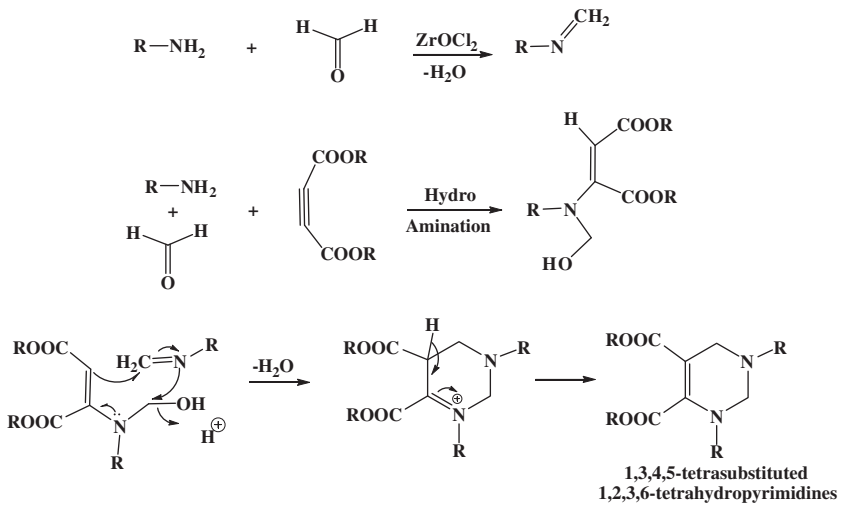
[a] Reaction condition: Aniline (2 mmol), diethyl acetylene dicarboxylate (1 mmol), (4 mmol) formaldehyde and $ZrOCl_2$ (10 mol %) in various solvents (5 mL) at reflux temperature.
[b] Isolated yields.

formaldehyde to form an *N*-methyleneaniline derivative with the loss of water molecule. In the next step of hydroamination, the amine, formaldehyde and the dicarboxylates reacts with each other to form phenyl amino maleate derivatives. The final step is the cycloaddition of *N*-methyleneaniline and phenyl amino maleate derivatives to form 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidine derivatives in good to excellent yields. The 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines were obtained within 15–20 min (Table 4) in high yields (85–91%). The reaction proceeded smoothly to furnish the desired products in good yields without substantial difference in time required for completion of reaction indicating the flexibility and sturdiness of the method. The practical applicability of this method was evaluated by using substituted amines and both dimethyl and diethyl acetylene dicarboxylates to afford the desired products in good yields in shorter reaction time (Table 4).

All the synthesized compounds were screened for in vitro antimicrobial activity. The antibacterial activity was evaluated against different bacterial strains such as *Staphylococcus aureus* (NCIM-2901), *Bacillus subtilus* (NCIM-2063) and *Escherichia coli* (NCIM-2256). Minimum inhibitory concentration (MIC, μg/mL) of antibacterial activity was determined using broth dilution method as per CLSI guidelines.[8–11] Ciprofloxacin and ampicillin were used as a standard drug for the comparison of antibacterial activity (Table 5). The antifungal activity was evaluated against different fungal strains such as *Candida albicans* (NCIM3471), *Aspergillus flavus* (NCIM539) and *Aspergillus niger* (NCIM1196). MIC values of antifungal activity were determined using standard agar dilution method as per CLSI guidelines.[8–11] Fluconazole and miconazole were used as standard drugs for the comparison of antifungal activity (Table 5). Dimethyl sulfoxide was used as solvent control.

From the antimicrobial data, it is observed that all the newly synthesized compounds shows good to moderate level of antibacterial and antifungal activity (Table 5). The antimicrobial activity data (Table 5), reveals that compounds (**4a**, **4b**, **4e** and **4f**), all of which having diethyl but-2-ynedioate, are found to be most active and potent as antimicrobial agents among the series. These compounds were found to be active against the tested fungal and bacterial strains, having MIC values (15–60 μg/mL for bacteria and 12.5–50 μg/mL for fungus) compatible with standard drugs, except for bacterium *S. aureus* which shows MIC values between (60 and 100 μg/mL). The compounds (**4h**, **4k** and **4l)** containing dimethyl but-2-ynedioate, shows reduced antibacterial properties compared to (**4b**, **4e** and **4f**), while the antifungal activity of compounds (**4h**, **4k** and **4l)** remained unchanged for the tested fungal strains. The compounds (**4d** and **4j)**, both formed by *m*-nitro aniline, are least active against tested bacterial strains (MIC 60–100 μg/mL) and fungal strains (MIC 100 μg/mL). The remaining compounds (**4c**, **4g** and **4i)** have intermediate antimicrobial activity compared to standard. In short the compounds **(4a, 4b**, **4e, 4f, 4h, 4k,** and **4l)** show promising antifungal activity indicating the future scope for optimization. The structure activity relationship of the series can be explained as,

(1) *Effect of alkyne substituent*: The diethyl but-2-ynedioate **(4a–f)** gave more potent molecules for the antibacterial activity. The replacement of diethyl but-2-ynedioate by dimethyl but-2-ynedioate **(4g–l)**, resulted in diminished antibacterial activity, however their antifungal properties remained unchanged. In short the diethyl but-2-ynedioate was more specific towards bacterial strains compared with dimethyl but-2-ynedioate.
(2) *Effect of Amine*: Introduction of phenyl ring on both nitrogen of pyrimidine ring **(4a, 4g)** shows promising antibacterial and antifungal activity compared to standard drugs. Introduction




**Scheme 2.** Plausible reaction mechanism for the synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines catalyzed by $ZrOCl_2$ in water.

**Table 4**
Synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives (**4a–l**) using $ZrOCl_2$ as catalyst in water[a]

| Entry | Amine | Alkynes | Time (min) | Melting point (°C) | Yield[b] (%) |
|---|---|---|---|---|---|
| **4a** | Aniline | Diethyl but-2-ynedioate | 15 | 234–236 | 90 |
| **4b** | *p*-Chloro aniline | Diethyl but-2-ynedioate | 17 | 263–265 | 88 |
| **4c** | *p*-Hydroxy aniline | Diethyl but-2-ynedioate | 15 | 219–221 | 90 |
| **4d** | *m*-Nitro aniline | Diethyl but-2-ynedioate | 18 | 237–239 | 89 |
| **4e** | 2 Amino pyridine | Diethyl but-2-ynedioate | 20 | 258–260 | 86 |
| **4f** | 2 Amino 6-methoxybenzothiazole | Diethyl but-2-ynedioate | 20 | 272–274 | 85 |
| **4g** | Aniline | Dimethyl but-2-ynedioate | 16 | 197–199 | 91 |
| **4h** | *p*-Chloro aniline | Dimethyl but-2-ynedioate | 18 | 259–261 | 87 |
| **4i** | *p*-Hydroxy aniline | Dimethyl but-2-ynedioate | 15 | 241–243 | 88 |
| **4j** | *m*-Nitro aniline | Dimethyl but-2-ynedioate | 20 | 250-252 | 91 |
| **4k** | 2 Amino pyridine | Dimethyl but-2-ynedioate | 19 | 235–237 | 89 |
| **4l** | 2 Amino 6-methoxybenzothiazole | Dimethyl but-2-ynedioate | 20 | 243–245 | 90 |

[a] Reaction condition: Substituted amines (2 mmol), substituted but-2-ynedioate (1 mmol), formaldehyde (4 mmol) and $ZrOCl_2$ (10 mol %) in water (5 mL) at reflux temperature.
[b] Isolated yields.

**Table 5**
Antimicrobial activity of the synthesized compounds (**4a–l**)

| Compound | MIC values[a] (μg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | *B. subtilus* | *S. aureus* | *E. coli* | *C. Albicans* | *A. Flavus* | *A. Niger* |
| **4a** | 25 | 60 | 15 | 50 | 25 | 25 |
| **4b** | 25 | 80 | 15 | 25 | 25 | 25 |
| **4c** | 40 | 100 | 50 | 75 | 50 | 50 |
| **4d** | 60 | 100 | 100 | 100 | 100 | 100 |
| **4e** | 20 | 100 | 60 | 25 | 12.5 | 25 |
| **4f** | 20 | 100 | 15 | 25 | 12.5 | 12.5 |
| **4g** | 30 | 90 | 60 | 50 | 25 | 25 |
| **4h** | 40 | 100 | 60 | 25 | 25 | 25 |
| **4i** | 80 | 90 | 80 | 75 | 50 | 50 |
| **4j** | 100 | 80 | 80 | 100 | 100 | 100 |
| **4k** | 40 | 80 | 60 | 25 | 12.5 | 25 |
| **4l** | 25 | 100 | 40 | 25 | 12.5 | 12.5 |
| Ciprofloxacin | 25 | 25 | 15 | — | — | — |
| Ampicillin | 50 | 50 | 50 | — | — | — |
| Fluconazole | — | — | — | 40 | 25 | 25 |
| Miconazole | — | — | — | 12.5 | 12.5 | 12.5 |

[a] Values are the average of three readings.

of *p-chloro* **(4b, 4h)** on phenyl ring decreases the antibacterial activity against bacterium *S. aureus* (MIC from 60 to 80 μg/mL for **(4b)** and for **(4h)** MIC from 90 to 100 μg/mL) whereas it increases antifungal activity against the fungus *C. albicans* (MIC from 50 to 25 μg/mL for both **4b** and **4h**), when compared to **(4a, 4g)** respectively. The introduction of *p-hydroxy* on phenyl ring **(4c, 4i)** reduces the overall antimicrobial activity of molecule on the tested strains compared to **(4a, 4g)** respectively. The introduction of *m-nitro* on phenyl ring **(4d, 4j)** further reduces the activity compared to **(4a, 4g)** respectively and were the least active molecule of the series.

(3) *Effect of heterocyclic amine*: The 2 amino pyridine **(4e, 4k)** and 2 amino 6-methoxybenzothiazole **(4f, 4l)** makes the molecule more potent compared to the respective unsubstituted analogues **(4a, 4g)**. This clearly highlights that the heterocycles help in increasing the antimicrobial activity.

(4) *Strain specific effects of heterocycles*: In compounds **(4f, 4l),** the replacement of pyridine ring by 6-methoxybenzothiazole ring, increased the activity against bacterium *E. coli* (MIC from 60 to 15 μg/mL) and fungus *A. niger* (MIC from 25 to 12.5 μg/mL), while the MIC values of this compounds for other bacterial and fungus strains remained unchanged. Thus it indicates that such a modifications are important in making the molecule more potent against the bacterium *E. coli* and fungus *A. niger*.

*Highlight of synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives*:[12]

The novelty and highlight in synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives is (i) the first report of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives, catalyzed by $ZrOCl_2$; (ii) evasion of cumbersome workup procedures; (iii) development of eco-friendly process by omission of organic solvents; (iv) Establishment of $ZrOCl_2$ as a versatile catalyst which is non-toxic and economically feasible to use in multicomponent reactions and also for hydroamination reactions; (v) Excellent yields in shorter reaction time making the process economically lucrative for industrial application; (vi) opening the horizon for the synthesis of series of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines with promising antibacterial and antifungal activity which are yet to be explored and exploring them for other biological applications.

In conclusion, we report a green, practical and facile approach for the synthesis of some new tetrahydropyrimidine analogues by multicomponent reaction of substituted amines, dialkyl acetylene dicarboxylates, and formaldehyde using $ZrOCl_2$ in water. The mild reaction conditions, shorter reaction time and promising antibacterial activity of (**4a**, **4b**, and **4f)** and antifungal activity (**4e**, **4f**, **4k** and **4l**) of the compounds compared to standard are the advantages of the present method. Lastly to develop potent antibacterial agent, diethyl but-2-ynedioate is better choice than dimethyl but-2-ynedioate, while for the development of antifungal agent both alkynes were equally pertinent.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.099.

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12. *General procedure for the synthesis of 1,3,4,5-tetrasubstituted 1,2,3,6-tetrahydropyrimidines derivatives (**4a–l**)*:
In a 50 ml round bottom flask, substituted amine (2 mmol), substituted but-2-ynedioate (1 mmol), formaldehyde (4 mmol) and $ZrOCl_2$ (10 mol %) in (5 mL) water. The reaction mixture was refluxed. The progress of reaction was monitored on TLC (30% ethyl acetate/n-hexane). The reaction got to completion within 15–20 min (Table 5), the product was obtained by filtration and dried in-vaccuo. The product was recrystallized using ethanol as solvent, with 85–91% yield (Table 5).