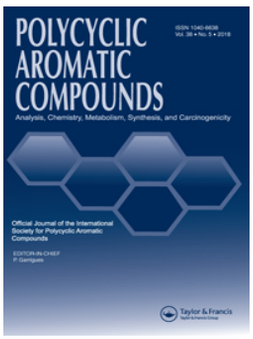

Polycyclic Aromatic Compounds

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An Expedient Four Component Synthesis of Substituted Pyrido-Pyrimidine Heterocycles in Glycerol:Proline Based Low Transition Temperature Mixture and Their Antioxidant Activity with Molecular Docking Studies

Priyanka P. Mohire[a], Dattatray R. Chandam[b], Ajinkya A. Patravale[c], Prafulla Choudhari[d], Vishram Karande[a], Jai. S. Ghosh[a], and Madhukar B. Deshmukh[a,e]

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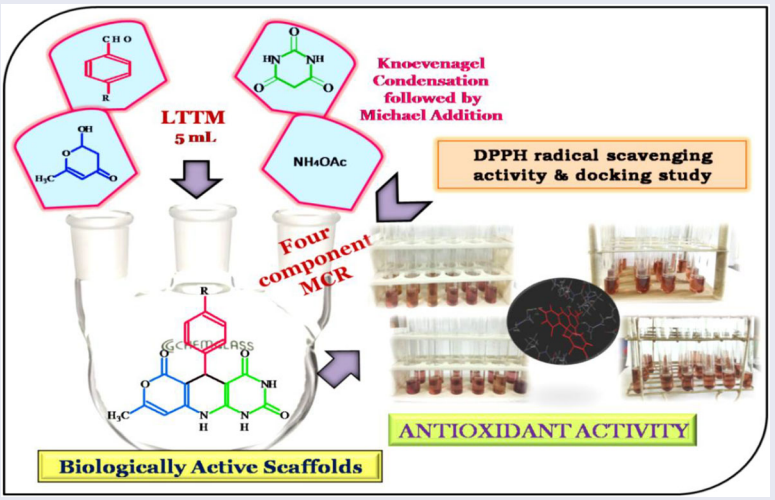
ABSTRACT

The present work describes an efficient and environmentally benign four component synthesis of structurally diverse substituted pyrido-pyrimidines involving the reaction of barbituric acid, 4-hydroxy 6-methyl 2-pyrone, an aromatic aldehyde and ammonium acetate in low transition temperature mixture (LTTM) (glycerol:proline: 1:1). The LTTM serves as green solvent which is an inexpensive, nontoxic and plays a dual function (reusable catalyst and solvent), and is found to be an excellent reaction endorsing medium. The present protocol offers some advantages such as easy work up, shorter reaction time, an excellent yield with higher atom economy, maximum reaction efficiency with an excellent green chemistry metrics. The synthesized compounds have been evaluated for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as well as molecular docking studies performed on myeloperoxidase enzyme. Tested scaffolds exhibits an excellent results of virtual screening and antioxidant activity.



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Introduction

Green solvents are one of the criteria in the green chemistry for the diversity-oriented synthesis of lead molecules.[1] Several solvents, such as water, ionic liquids, deep eutectic solvents (DESs), low transition temperature mixtures (LTTMs), are generally considered as green.[2] Among these, the synthesis process of Ionic Liquids is complex and expensive and difficult to purify.[3] In recent years, DES first introduced by Abbott et al.,[4,5] low melting mixtures first developed by Konig and coworkers[6] and LTTMs first proposed by Kroon and coworkers have similar physical properties.[7] DES or LTTMs are simply combinations of two or three safe and inexpensive components to form eutectic mixture through hydrogen bond interactions. The general properties of DES or LTTMs are low vapor pressure, water compatibility, biodegradability and non-inflammability, which make them suitable as green solvent.[8] Various DES or LTTMs have been employed for a variety of applications.[9–15]

Pyrido[2,3-*d*]pyrimidine heterocycles (also known as triazanaphthalenes) have received considerable attention due to their wide range of bioactivity, and they have been isolated from the wing pigments of European butterflies in the nineteenth century.[16,17] Pyrido[2,3-*d*]pyrimidines have been reported as the most abundant isomer in the literature because of broad-spectrum biological activities such as, anticancer,[18,19] antimicrobial,[20–22] anti-inflammatory and analgesic,[23,24] antiviral,[25–27] antihypertensive,[28] potent inhibitor of dihydrofolate reductase (DHFR),[29] antioxidant,[30] which is an important target site in various parasitic diseases, tyrosine kinase inhibitor,[31,32] antihistaminic,[33] antileishmanial,[34] diarrhea,[35] also molluscicidal agents against *Biomphalaria alexandrina* snails,[36] insecticidal agents.[37]

Thus, pyridopyrimidines fused with another active heterocyclic ring then become more active hetero-nucleus in the field of pharmaceutical and other fields with a wide spectrum of biological activities with several applications.[32,38,39]

Multicomponent reactions (MCRs) are of great importance for the synthesis of numerous heterocyclic moieties in organic and medicinal chemistry.[40] MCRs have become powerful tool than the conventional pathways for any organic synthesis. New and diverse heterocycles have been developed through MCRs in combinatorial chemistry.[41] MCRs represent an eco-friendly route with a high atom economy. Various MCRs have been reported in designer solvents such as DES or LTTM.[42] Pyridopyrimidine moiety with pyran nucleus through MCR has been reported via three-component reaction by Esmaeili et al.[43]

Numerous methods have been studied for the synthesis of pyrido[2,3-*d*]pyrimidine nucleus that have been appeared in the literature. However, these methodologies have various disadvantages such as the use of hazardous solvents, longer reaction time, expensive catalyst and generation of waste products.

Myeloperoxidase (MPO) is a peroxidase enzyme released in neutrophils and monocytes. MPO produces hypochlorous acid in their antimicrobial action that creates surrounding within phagolysosomes of neutrophils, which leads to microbial killing and overproduction of hypochlorous acid and causes an oxidative injury in host tissues. Antioxidants protect those organisms.[44,45]

Antioxidants are one of the chemicals which scavenge free radicals and help to prevent and control several diseases. Reactive oxygen species (ROS) serve as the free radicals produced through various physiological and biochemical processes in human body.[46] Antioxidants terminate chain reactions by removing free radical intermediates and inhibit other oxidation reactions by getting themselves oxidized. They play an important role in preventing chronic diseases. Naturally occurring substances in higher plants exhibit antioxidant activity.[47] Various numbers of nitrogen and oxygen containing heterocyclic organic compounds have significant antioxidant activity.[48,49] The synthesized derivatives were screened for antioxidant properties using DPPH radical scavenging activity. DPPH is a stable free radical, at room temperature, which forms violet color in an organic solvent like ethanol. In the presence of an antioxidant molecule, it can be reduced giving the colorless ethanolic solution hence an easy way to evaluate antioxidant activity.

In view of this goal for the development of environmentally benign protocols for the proficient preparation of diverse heterocycles, and to remove all the drawbacks of the reaction as well as to set up eco-friendly method, we have explored LTTM[50] as a potential solvent for the synthesis of 5-(aryl)-8-methyl-5,10-dihydro-2*H*-pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine-2,4,6(1*H*,3*H*)-trione using glycerol:proline (LTTM) through one-pot MCR (Scheme 1) and performed *in vitro* antioxidant activity by using DPPH radical scavenging assay. Further, the molecular docking studies have been carried out on MPO enzyme.

Result and discussion

Chemistry

One-pot MCRs were investigated for the synthesis of a series of substituted pyridopyrimidine derivatives using LTTM (glycerol:proline: 1:1) through an efficient procedure. We initially investigated the reaction of barbituric acid (1 mmol, 0.128 g) and 4-chloro benzaldehyde (1 mmol, 0.140 g), 4-hydroxy 6-methyl 2-pyrone (1 mmol, 0.126 g) and NH_4OAc (1 mmol, 0.077 g), using different reaction conditions and yielded 5-(4-chloro)-8-methyl-5,10-dihydro-2*H*-pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine-2,4,6(1*H*,3*H*)-trione.

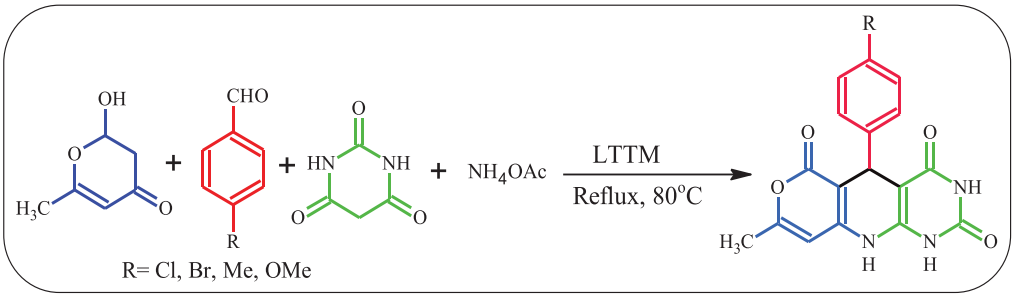
To optimize the reaction condition, the model reaction was carried out in a catalyst-free and solvent-free condition, and also by using water as a solvent at room temperature but reaction proceeds up to the formation of Knoevenagel condensation product (Table 1, entries 1–3). Further, we tried to check the solvent efficiency, for that the reaction was carried out in different polar and non-polar solvents. However, the representative reaction gav a poor yield. The yield of the product in water and mixture of water:ethanol was poor under refluxed condition (Table 1, entries 4–6). We screened the reaction in different solvents such as methanol, ethanol, acetonitrile, THF, etc. up to 30–360 min under refluxed condition but the yield of the reaction was observed to be poor (Table 1, entries 7–9).

Some LTTM or DESs were screened to test the efficiency to optimize the reaction conditions. The LTTM and DES as a solvent gave good yield as compared to another solvent (Table 1). As presented in Table 2, we carried out the reaction in different acid and base catalysts (20 mol %), however, desired results were not obtained and the yield of the product was not satisfactory.

After the optimization of the reaction conditions of the solvent and catalyst, it is clear from Tables 1 and 2 that the desired product pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine was obtained in an excellent yield in glycerol:proline LTTM (1:1) (Table 2, entry 6) indicating the suitability of environmentally viable reaction endorsing medium.

The structures of the compound code **4d$_4$** were confirmed based on spectroscopic data. The infrared showed ν_{max} 2997 cm^{-1} due to ([sbond]NH) and other bands at 1718, 1674, 1634 cm^{-1} due to the presence of a carbonyl group. The ^{1}H-NMR spectrum showed a singlet at δ 5.703 due to benzylic ([sbond]CH) proton which confirms the cyclization. The signals that appeared between δ 6.9 and 7.12 ppm indicate the aromatic protons. The signals that appeared at δ 5.88 and 9.3 represent ([sbond]NH) protons. In the 13C-NMR spectrum, the signal appeared at δ 20.7, 41.0, 79.7, 103.7, 109.2, 128.8, 128.9, 130.5, 130.9, 131.3, 140.3, 149.7, 150.5, 151.1 ppm indicating the presence of aromatic carbons. Characteristic carbonyl carbons appeared at δ 162.6, 163.8, 164.2 ppm in CMR. The mass spectrum of the same compound showed a molecular ion peak at *m/z* 357 confirming the proposed structure. After the successful synthesis of compound **4d$_4$**, we made attempts to develop a convenient, eco-friendly protocol based on green chemistry principles.

With the promising results using optimized conditions, a series of pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives (as presented in Table 3) were synthesized in an excellent yield starting with different aromatic aldehydes having electron-donating as well as electron-withdrawing



Scheme 1. Glycerol:proline (1:1) LTTM mediated synthesis of Pyrano-[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives.

groups. Aromatic aldehydes with electron-withdrawing groups require less time with high yield as compared to electron-donating groups.

It is noteworthy to reveal that this methodology worked well for hetero-aromatic aldehydes (Table 3). A plausible mechanism for the synthesis of 5-(aryl)-8-methyl-5,10-dihydro-2*H*-pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine-2,4,6(1*H*,3*H*)-trione (**5**) has been depicted in Scheme 2. The reaction may proceed through Knoevenagel condensation of the Barbituric acid (**1**) with an aromatic aldehyde (**2**) in (glycerol:proline) LTTM. The 4-hydroxy 6-methyl 2-pyrone (**3**) then undergoes subsequent aza-Michael addition followed by intramolecular cyclization with the carbonyl group of barbituric acid. The NH_4OAc (**4**) may act as an amine source in the reaction mechanism to give Michael adduct which undergoes dehydration to afford the desired product (Scheme 2). The role of LTTM as a catalyst or reaction promoting medium is still not clear, it may play dual role as a solvent as well as a catalyst. The hydrogen bonding nature of LTTM may activate the carbonyl group as depicted in plausible mechanism. The nature of aldehyde leads to the yield of the condensation product. Electron donating substituents will give the desired product with almost quantitative yields, while electron withdrawing substituents will give good yield of the product.

Virtual screening of synthesized derivatives

Docking simulations

Molecular docking was performed to ascertain the mode of action of pyrido[2,3-*d*]pyrimidine derivatives. Molecular docking was performed using MPO enzyme, which plays a vital role in the immune systems via the production of ROS. ROS have been identified as the critical factor in Parkinson's, Alzheimer's and cancer and kidney damage. All the synthesized derivatives were found to bind excellently with MPO enzyme. The binding energy of all the compounds ranges from −71.56 to −55.64 kcal/mol. Most active molecule **4d$_3$** was found to interact with human MPO (Figure 1) with the formation of hydrogen bond interactions with ARG323 and ARG31 and hydrophobic interactions with ALA28, PHE29 and VAL30. A number of Van der Waals interactions were also observed with ALA28, PHE29, VAL30, ARG31, ASN162, ASP321 and ARG323. Derivative **4d$_{12}$** was found to interact with protein via formation of a hydrogen bond with ARG323 and ARG31 and hydrophobic interactions with ALA28, PHE29, VAL30 and ILE160. Van der Waals interactions were also observed with VAL30, ARG31, ASN162, ASP321 and ARG323. The compound code **4d$_{13}$** was found interacting with protein via formation of hydrogen bond with ASN162 and hydrophobic interactions with ALA28, PHE29, VAL30 and PRO34. (Remaining figures of docking are included in the Supporting Information).

ADME prediction

ADME prediction of the synthesized molecules was carried out using http://www.swissadme.ch. All the molecules showed excellent ADME parameters, which indicated their good oral

Table 1. Optimization of solvent for the synthesis of pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine (**4d$_4$**).[a]

Sr. no.	Solvent	Temp. condition (°C)	Reaction time (min.)	Yield[b] (%)
1.	Solvent free	30	360	–[c]
2.	Solvent free	80	360	–[c]
3.	Water	30	360	–[c]
4.	Water	80	240	42
5.	Ethanol	80	240	48
6.	Water:ethanol (1:1)	80	360	52
7.	Methanol	80	120	53
8.	Acetonitrile	80	120	40
9.	THF	80	180	38
10.	Glycerol	80	120	58
11.	Choline chloride:oxalic acid (1:1)	80	120	58
12.	Choline chloride:oxalic acid (1:2)	80	120	48
13.	**Glycerol:proline (1:1)**	**80**	**35**	**92**

[a]Reaction condition: Barbituric acid (1 mmol) and 4-chloro benzaldehyde (1 mmol), 4-hydroxy 6-methyl 2-pyrone (1 mmol) and NH_4OAc (1 mmol) heated at 80 °C in 5 mL of respective solvent.
[b]Yields refer to pure isolated products.
[c]Knoevenagel condensation product.

Table 2. Optimization of catalyst for the synthesis of pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine (**4d$_4$**).[a]

Sr. no.	Catalyst	Catalyst load (mol %)	Temp. conditions (°C)	Time (min)	Yield[b] (%)
1.	NaOH	20	80	180	44
2.	DBN	20	80	180	46
3.	PTSA	20	80	180	50
4.	Acetic acid	20	80	120	60
5.	Proline	20	80	120	68
6.	**Glycerol:proline (1:1)**	–	80	**35**	**92**

[a]Reaction condition: Barbituric acid (1 mmol) and 4-chloro benzaldehyde (1 mmol), 4-hydroxy 6-methyl 2-pyrone (1 mmol) and NH_4OAc (1 mmol) heated at 80 °C in 5 mL of respective solvent.
[b]Yields refer to pure isolated products.

absorption. All the molecules found to have in Lipinski parameters. The results of ADME prediction are given in Table 4.

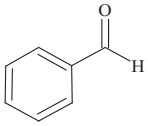
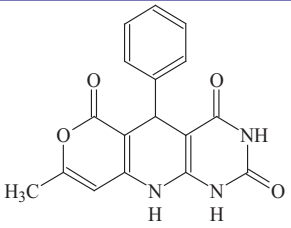
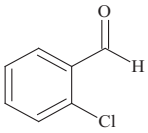
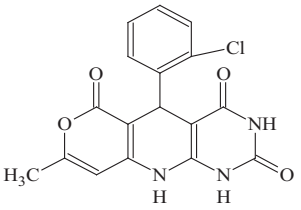
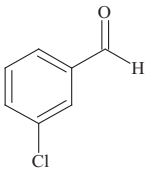
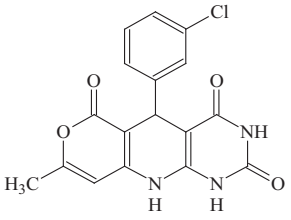
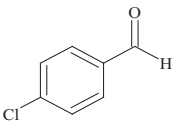
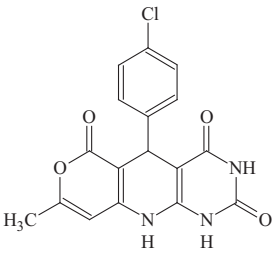
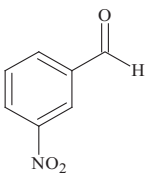
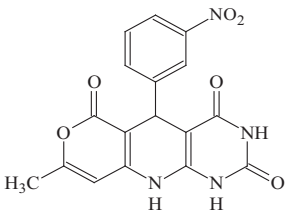
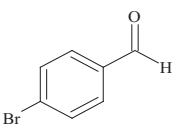
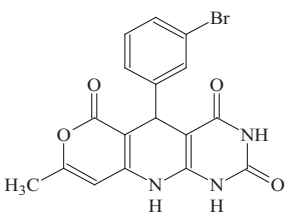
Antioxidant activity results

The DPPH scavenging activity gives the information about the antioxidant property of the newly synthesized pyrido[2,3-*d*]pyrimidine derivatives with stable-free radical. The delocalization of electron makes the solution deep violet color which is characterized by an absorption band in ethanol solution at about 517 nm. When DPPH solution is combined with the other substrate, donates a hydrogen atom, which gives rise to the reduced form, through the disappearance of this violet color. Free radical scavenging activity of the test compounds **4d$_1$** to **4d$_{13}$** was done by DPPH radical scavenging method. The results presented in graphical format reveals that the compound code **4d$_3$**, **4d$_5$**, **4d$_{12}$**, **4d$_{13}$** of pyrido[2,3-*d*]pyrimidine derivatives possessed significant activity at 50 µg/ml and 100 µg/ml while other showed moderate activity as compared to standard compound. While compound code **4d$_3$**, **4d$_{12}$**, and **4d$_{13}$** exhibited an excellent antioxidant activity (Figure 2). (Results of Antioxidant Activity are mentioned in the Supporting Information)

Green metric calculations

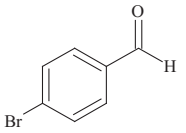
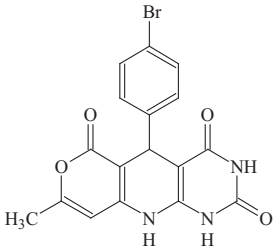
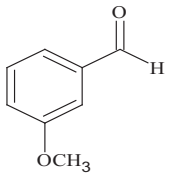
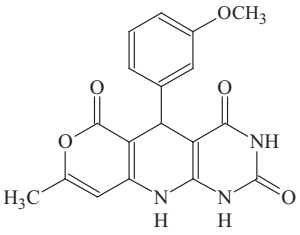
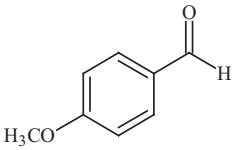
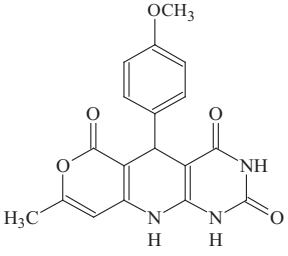
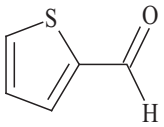
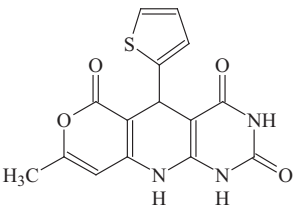
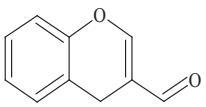
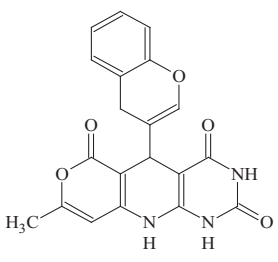
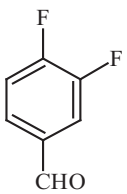
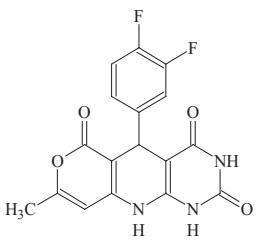
Further, we performed green metrics calculations such as mass intensity (MI), reaction mass efficiency (RME), carbon efficiency (CE) and atom economy (AE) and *E* factor which explain the efficiency of the protocol in terms of green chemistry.[51,52]

Table 3. Synthesis of pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine using glycerol:proline (LTTM) (entries **4d$_1$ to 4d$_{13}$**)[a].

Entry no.	Aldehyde	Product[b]	Time (min)	Yield (%)[c]	M.p. (°C)
4d$_1$			35	90	282–285
4d$_2$			40	84	257–260
4d$_3$			45	88	238–241
4d$_4$			35	92	248–250
4d$_5$			45	86	278–280
4d$_6$			50	90	260–263
4d$_7$			45	92	280–284

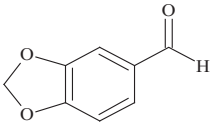
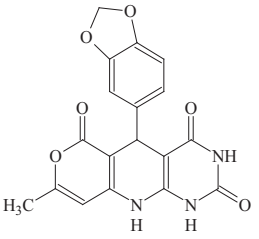
(*continued*)

Table 3. Continued.

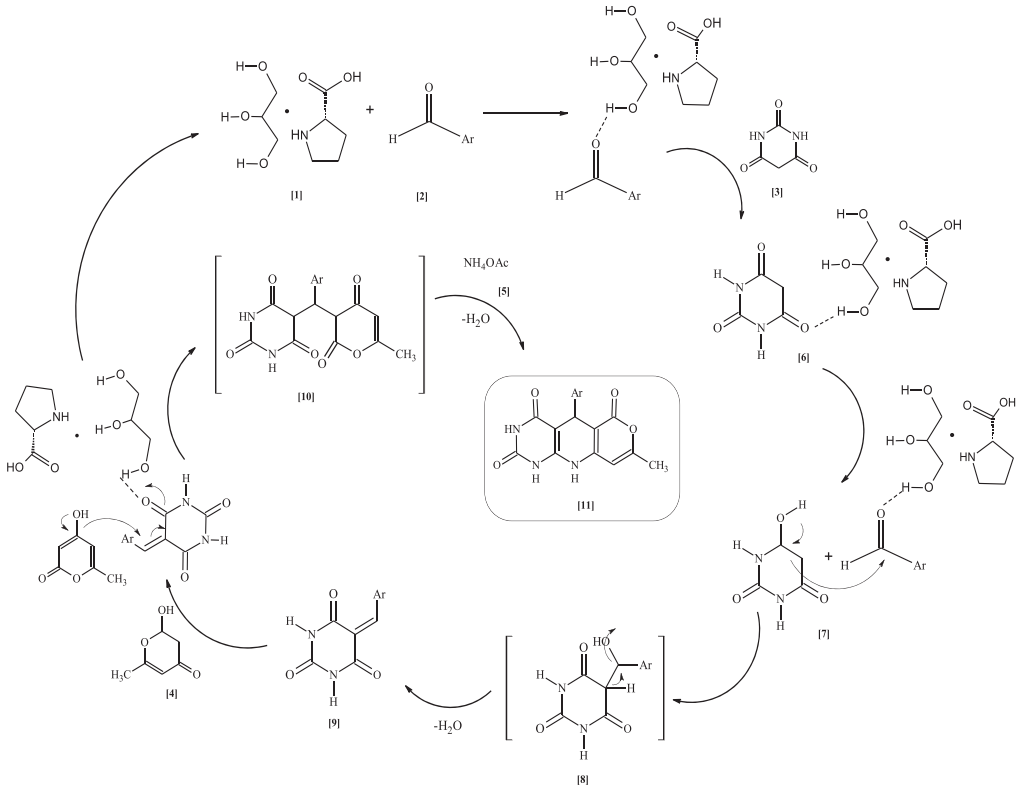
Entry no.	Aldehyde	Product[b]	Time (min)	Yield (%)[c]	M.p. (°C)
					
4d$_8$			45	82	278–280
4d$_9$			35	92	274–277
4d$_{10}$			45	86	254–256
4d$_{11}$			50	90	>300
4d$_{12}$			40	80	285–288

(*continued*)

Table 3. Continued.

Entry no.	Aldehyde	Product[b]	Time (min)	Yield (%)[c]	M.p. (°C)
$4d_{13}$			45	92	259–260

[a]Reaction condition: Barbituric acid (1 mmol) and 4-chloro benzaldehyde (1 mmol) 4-hydroxy 6-methyl 2-pyrone (1 mmol) and NH_4OAc (1 mmol) heated at 80 °C in 5 mL of respective solvent.
[b]Yields refer to pure isolated products.
[c]Products prepared for first time.



Scheme 2. Mechanistic pathway of 5-(aryl)-8-methyl-5,10-dihydro-2*H*-pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine-2,4,6(1*H*,3*H*)-trione.

In ideal condition MI ≈ 1%, RME ≈ 100%, %CE ≈ 100 and %AE ≈ 100 and *E*-factor ≈ 0 is expected. To prove the greenness of the present protocol, the green metrics were calculated using literature procedures.[53–56]

Figure 3 represents the green metric calculations of the synthesized compounds. The high yield of the product shows that the significant RME values and moderate yield generate moderate RME values. In addition to this, all the carbon atoms in the reactants are present in the product and show excellent values of % CE. MI values and *E* factor of the reaction are very close to the

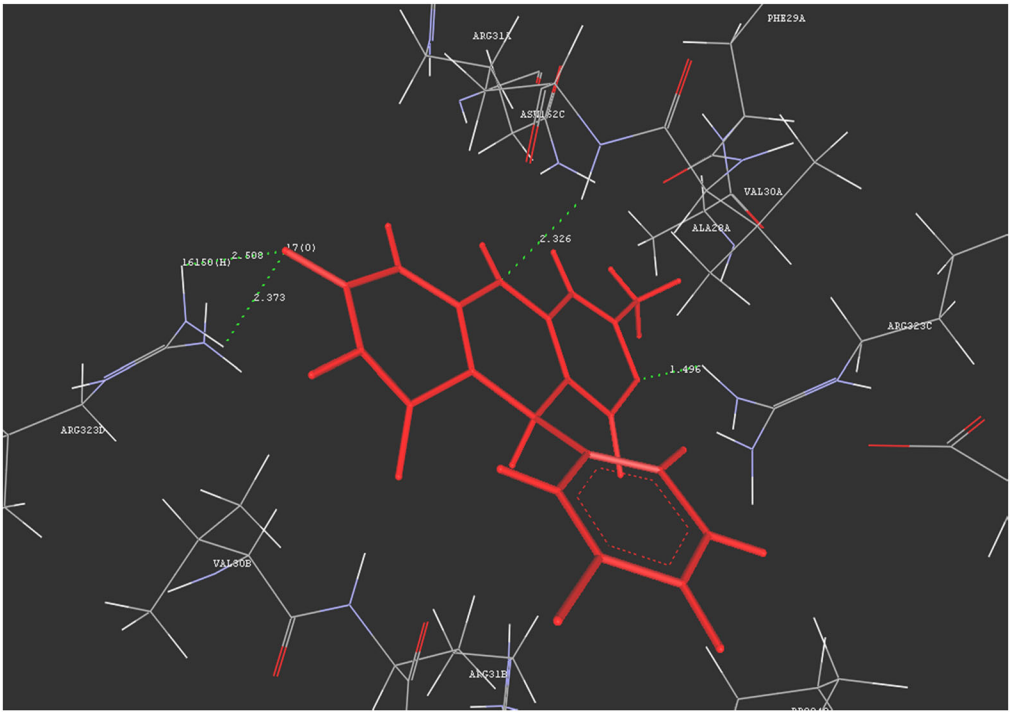


Figure 1. Significant interaction of molecule **$4d_3$** with MPO.

Table 4. Lipinski parameters of synthesized compounds.

Compd. code	No. of H-bond acceptors	No. of H-bond donors	TPSA	iLOGP	GI absorption	Bioavailability score
$4d_1$	4	3	107.96	1.57	High	0.55
$4d_2$	4	3	107.96	2.04	High	0.55
$4d_3$	4	3	107.96	1.82	High	0.55
$4d_4$	4	3	107.96	1.81	High	0.55
$4d_5$	6	3	153.78	0.97	Low	0.55
$4d_6$	4	3	107.96	1.93	High	0.55
$4d_7$	4	3	107.96	1.88	High	0.55
$4d_8$	5	3	117.19	1.84	High	0.55
$4d_9$	5	3	117.19	1.8	High	0.55
$4d_{10}$	4	3	136.2	1.54	High	0.55
$4d_{11}$	5	3	117.19	1.9	High	0.55
$4d_{12}$	6	3	107.96	1.69	High	0.55
$4d_{13}$	6	3	126.42	1.81	High	0.55

ideal values. The percent atom economy of each scaffold indicates maximum conversion of starting materials into product with minimum waste exclusion. Mass of catalyst is excluded as it is recyclable. All these calculations are represented in graphical state that, the present protocol is very close to the ideal condition along with reusability of LTTM on the basis of green chemistry. (Table of green metrics calculations included in the Supporting Information.)

Reusability of LTTM

As shown in Figure 4, we studied the reusability of LTTM as a catalyst for the same reaction. It is an important aspect of green and sustainable chemistry. In the present study, after completion of the reaction under optimized condition, 10 ml water was added to the reaction mixture and the crude reaction product was separated by simple filtration. The LTTM was recovered by

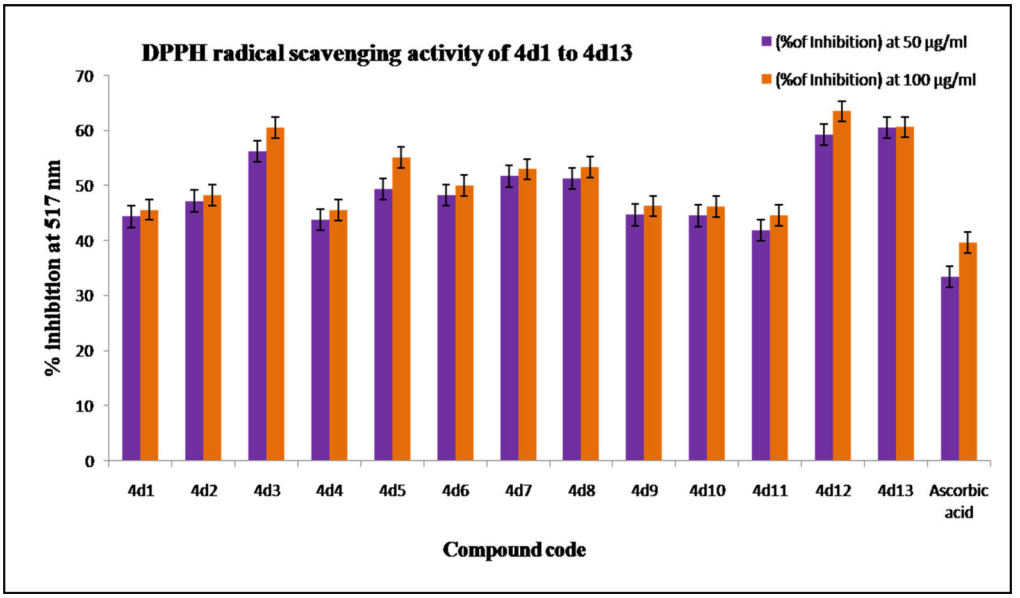


Figure 2. Antioxidant activity of pyrido[2,3-*d*]pyrimidine derivatives.

evaporating the water at 80 °C under vacuum. The recovered LTTM was successively reused for the next reaction and again recycled.

Experimental section

All the chemicals were purchased from Alfa Aesar and Spectrochem (PVT. Ltd, Mumbai, India), Sigma Aldrich and used without purification. The reaction was monitored by TLC. The desired structures of the synthesized compounds were confirmed by their relevant spectral data. The melting points were determined in open glass capillary method and are uncorrected. The compounds were confirmed by IR, ^{1}H NMR and 13C NMR. The IR spectra were recorded on a JASCO FT-IR 4600 spectrum spectrophotometer and the values are expressed as ν_{max} cm^{-1}. The ^{1}H NMR and 13C NMR spectra were recorded on Bruker Spectrospin Avance II-300 MHz and 75 MHz spectrophotometer relative to TMS as an internal standard using Dimethyl Sulfoxide (DMSO-d_6) as a solvent.

General synthetic procedure for the preparation of glycerol:proline (1:1) LTTM

We described the procedure for the preparation of LTTM in our previous article,[50] by selecting glycerol as a hydrogen bond donor and proline as a hydrogen bond acceptor. A mixture of glycerol (100 mmol, 9.2 g) and proline (100 mmol, 11.5 g) in the ratio 1:1 was heated at 80 °C with continuous stirring for 30 min (Scheme 3). The resulting DES (LTTM), dark yellowish viscous liquid with an excellent atom economy, was subsequently allowed to cool at room temperature and used for the synthesis of pyrido[2,3-*d*]pyrimidine derivatives without further purification.

General synthetic procedure for the synthesis of pyrano[3′,4′:5,6]pyrido[2,3-d]pyrimidine derivatives

A mixture of barbituric acid (1 mmol 0.128 g) and 4-chlorobenzaldehyde (1 mmol, 0.140 g), was taken in a 50-ml round bottomed flask and stirred at room temperature to get the Knoevenagel

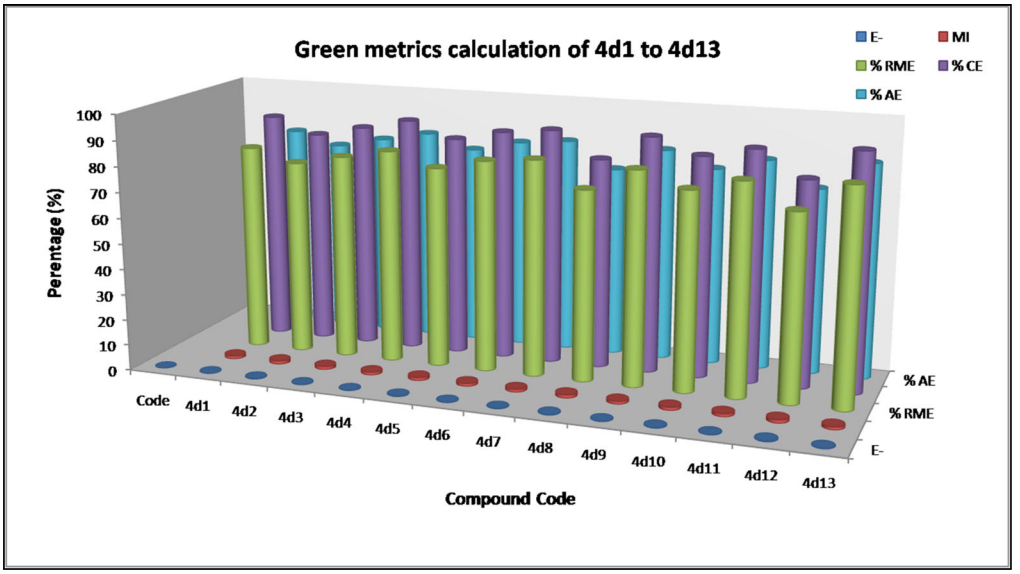


Figure 3. Green metrics calculations of synthesized pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives.

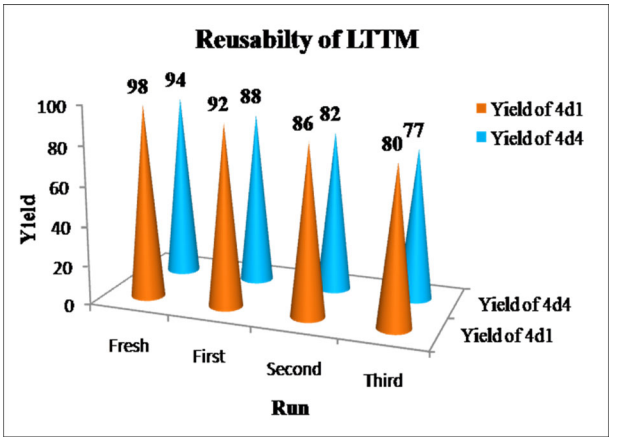
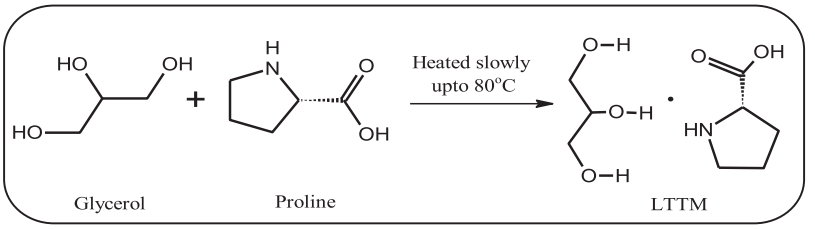


Figure 4. Reusability of LTTM.

product, monitored by TLC. Then 4-hydroxy 6-methyl 2-pyrone (1 mmol, 0.126 g) and NH_4OAc (1 mmol, 0.077 g) were added and the mixture continued at reflux using LTTM (5 ml) for 25 min. The progress of the reaction was monitored by TLC using petroleum ether-ethyl acetate (8:2 vol/vol). After completion, the reaction mixture was cooled to room temperature. The crude product formed was filtered, washed with hot ethanol and diethyl ether. Finally, the crude product was recrystallized from ethanol to get pure product (Scheme 1).

Molecular docking analysis

Molecular docking was performed to check the mechanism of actions of the synthesized derivative for antioxidant potential. Crystal structure of human MPO, (PDB code: 1DNU, 1.9 Å resolution) was utilized for the docking analysis downloaded from free protein database www.rcsb.org. Prior to the docking analysis, ligand structure was prepared via using molecule builder module and subsequently optimized via application of Merck molecular force field. Protein structure



Scheme 3. Preparation of glycerol:proline (1:1) LTTM.

was prepared using biopredicta module of the V life MDS 4.3. Protein structure was refined via removal of water molecule and addition of hydrogen atoms so that it will regain its original geometry. Grip based docking simulations was performed keeping macromolecules in rigid conformation and micromolecules in flexible conformation.[57–60]

ADME prediction

ADME plays an important role in the drug action as these properties govern the pharmaco-kinetic issues of the molecules.[61–64] The ADME prediction of the synthesized molecules was carried out using http://www.swissadme.ch.

Experimental procedure of DPPH radical scavenging activity

The DPPH radical scavenging activity[65] was performed for determination of antioxidant activity of the synthesized pyrido[2,3-*d*]pyrimidine scaffolds. About 0.004% wt/vol DPPH solution was prepared in methanol. Standard ascorbic acid was prepared in the concentration of 10 mg/100 ml from the prepared stock solution. About 20, 40, 60, 80 and 100 μl of this solution were taken in test tubes, respectively. Concentrations of the tested compounds were prepared in DMF solvent. The final concentrations are 50 µg/ml and 100 µg/ml. In each test tube, 1 ml of the sample concentration was added and to these same test tube 3 ml of freshly prepared DPPH solution was added. Then the test tubes were incubated in dark for 30 min and after that absorbance were measured at λ_{max} 517 nm against blank. The control was prepared as above without test compound. The absorbance was recorded of control after 30 min. This protocol was performed in triplicate. The antioxidant activity was calculated as the percentage inhibition of DPPH radical. The DPPH radical scavenging activity was calculated using the following formula

$$DPPH scavenging activity\ (\%) = [(A_c - A_t)/A_c] \times 100$$

where,

A_c = absorbance of the control and

A_t = Absorbance of the tested compounds or standards.

Ascorbic acid at the concentration range of 100 μg/ml was used as a standard.

(The results obtained from antioxidant assay have been presented in tabular form in the Supporting Information.)

Spectroscopic data of the synthesized compounds

8-Methyl-5-phenyl-5,10-dihydro-2H-pyrano [3′,4′:5,6] pyrido[2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_1$)

White powder, yield 90%; m.p. 282 °C; IR (ν_{max}): 3019.18 ([sbond]NH), 1720.19 (C[dbond]O), 1699.94 (C[dbond]O), 1667.16 (C[dbond]O), 1634.38 (C[dbond]C) cm^{-1}; ^{1}H NMR (300 MHz,

DMSO-d_6): δ, 2.058–2.096 (d, 3H, CH_3, $J = 7.8$ Hz), 5.838 (s, 1H, [sbond]CH), 6.940–6.967 (d, 2H, Ar[sbond]H, [sbond]NH, $J = 7.8$ Hz), 7.161–7.189 (d, 4H, Ar[sbond]H, $J = 7.8$ Hz), 9.883 (s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.20, 33.06, 90.08, 99.98, 101.72, 103.22, 103.63, 105.14, 128.05, 128.65, 130.07, 141.06, 141.96, 151.98, 160.11, 160.49, 165.96, 167.56, 170.30, 173.63 ppm; mass (*m/z*): 323.3028 (M^+). Anal. calcd. For $C_{17}H_{13}N_3O_4$: C, 63.16; H, 4.05; N, 13.00%. Found: C, 63.10; H, 3.98; N, 12.88%.

*5-(2-Chlorophenyl)-8-methyl-5,10-dihydro-2*H*-pyrano [3′,4′:5,6]pyrido [2,3-*d*] pyrimidine-2,4,6(1*H*,3*H*)-trione (4d$_2$)*

White powder; yield 84%; m.p. 257 °C; IR (ν_{max}): 2971.77 ([sbond]NH), 2901.38 ([sbond]NH), 2817.49 ([sbond]NH), 1738.51 (C[dbond]O), 1664.27 (C[dbond]O), 1574.59 (C[dbond]C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.071 (s, 3H, CH_3), 4.165 (s, 1H, [sbond]NH), 5.662 (s, 1H, [sbond]CH), 7.003–7.277 (q, 4H, Ar[sbond]H, $J = 7.8$ Hz), 7.925 (s, 1H, Ar[sbond]H), 9.360 (s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.39, 33.63, 56.60, 88.24, 103.54, 103.67, 125.94, 126.93, 129.56, 130.53, 133.23, 141.89, 151.78, 158.89, 165.55, 166.15, 169.72 ppm; mass (*m/z*): 357.7478 (M^+). Anal. calcd. For $C_{17}H_{12}ClN_3O_4$: C, 65.92; H, 3.60; N, 11.52%. Found: C, 65.88; H, 3.58; N, 11.45%.

*5-(3-Chlorophenyl)-8-methyl-5,10-dihydro-2*H*-pyrano [3′,4′:5,6]pyrido [2,3-*d*] pyrimidine-2,4,6(1*H*,3*H*)-trione (4d$_3$)*

White powder; yield 88%; m.p. 238 °C; IR (ν_{max}): 2973.70 ([sbond]NH), 2896.56 ([sbond]NH), 2816.53([sbond]NH), 1620.88 (C[dbond]O), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.054–2.094 (d, 3H, CH_3, $J = 7.8$ Hz), 5.712 (s, 1H, [sbond]CH), 5.810–5.833 (d, 1H, [sbond]NH, $J = 7.8$ Hz), 6.937–6.964 (d, 2H, Ar[sbond]H, $J = 7.8$ Hz), 7.159–7.187 (d, 3H, Ar[sbond]H, $J = 7.8$ Hz), 9.837 (s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.22, 33.05, 90.05, 99.98, 101.70, 103.20, 103.20, 103.64, 105.15, 128.04, 128.66, 128.78, 130.04, 142.03, 151.96, 160.04, 160.04, 160.43, 160.95, 167.51, 167.84, 170.29, 173.06 ppm; mass (*m/z*): 357.7478 (M^+). Anal. calcd. For $C_{17}H_{12}ClN_3O_4$: C, 65.92; H, 3.60; N, 11.69%. Found: C, 65.88; H, 3.58; N, 11.64%.

*5-(4-Chlorophenyl)-8-methyl-5,10-dihydro-2*H*-pyrano [3′,4′:5,6] pyrido [2,3-*d*] pyrimidine-2,4,6(1*H*,3*H*)-trione (4d$_4$)*

White powder; yield 92%; m.p. 248 °C; IR (ν_{max}): 2997.8 ([sbond]NH), 1718.26 (C[dbond]O), 1673.91(C[dbond]O), 1634.38 (C[dbond]O), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.094 (s, 3H, CH_3), 5.703 (s, 1H, [sbond]CH), 5.884 (s, 1H, [sbond]NH), 6.995–7.139 (m, 5H, Ar[sbond]H), 9.628 (s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.39, 29.39, 33.18, 78.79, 79.23, 79.43, 79.67, 89.49, 101.53, 103.25, 103.73, 105.30, 127.78, 128.86, 129.57, 129.78, 131.61, 143.17, 151.67, 159.67 ppm; mass (*m/z*): 357.7478 (M^+). Anal. calcd. For $C_{17}H_{11}Cl_2N_3O_4$: C, 52.06; H, 2.83; N, 10.71%. Found: C, 52.02; H, 2.78; N, 10.64%.

*8-Methyl-5-(3-nitrophenyl)-5,10-dihydro-2*H*-pyrano [3′,4′:5,6] pyrido[2,3-*d*] pyrimidine-2,4,6(1*H*,3*H*)-trione (4d$_5$)*

White powder; yield 86%; m.p. 278 °C; IR (ν_{max}): 2971.77 ([sbond]NH), 2897.52 ([sbond]NH), 2817.49 ([sbond]NH), 1739.48 (C[dbond]O), 1668.12 (C[dbond]O), 1637.27 (C[dbond]O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.125 (s, 3H, CH_3), 5.830 (s, 1H, [sbond]CH), 6.005–6.035 (d, 1H, Ar[sbond]H, [sbond]NH, $J = 9.0$ Hz), 7.455–7.506 (t, 3H, Ar[sbond]H), 7.939–7.963 (d, 3H, Ar[sbond]H, $J = 7.2$ Hz), 9.862 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.42, 33.71, 88.26, 89.11, 90.78, 99.98, 101.48, 102.61, 103.69, 120.51, 121.37, 129.60, 134.14,

147.10, 148.07, 151.14, 151.64, 159.85, 165.85, 166.60, 170.17 ppm; mass (*m/z*): 368.3003 (M^+). Anal. calcd. For $C_{17}H_{12}N_4O_6$: C, 55.44; H, 3.28; N, 15.21%. Found: C, 55.40; H, 3.26; N, 15.16%.

5-(3-Bromophenyl)-8-methyl-5,10-dihydro-2H-pyrano[3′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6(1H,3H)-trione (4d$_6$)

White powder; yield 90%; m.p. 260 °C; IR (ν_{max}): 2971.77 ([sbond]NH), 2882.09 ([sbond]NH), 2815.56 ([sbond]NH), 1740.44 (C[dbond]O), 1614.13 (C[dbond]C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 2.086 (s, 3H, CH_3), 5.725 (s, 1H, [sbond]CH), 5.813 (s, 1H, Ar[sbond]H), 6.931–6.966 (d, 2H, Ar[sbond]H, [sbond]NH, $J = 10.5$ Hz), 7.190–7.218 (d, 3H, Ar[sbond]H, $J = 8.4$ Hz), 9.586 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.50, 33.76, 56.18, 90.06, 103.26, 103.90, 105.18, 114.18, 129.94, 133.78, 150.82, 156.44, 158.88, 159.78, 164.20, 168.48, 170.02 ppm; mass (*m/z*): 402.1988 (M^+). Anal. calcd. For $C_{17}H_{12}BrN_3O_4$: C, 50.77; H, 3.01; N, 10.45%. Found: C, 50.74; H, 2.98; N, 10.40%.

5-(4-Bromophenyl)-8-methyl-5,10-dihydro-2H-pyrano [3′,4′:5,6] pyrido [2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_7$)

White powder; yield 92%; m.p. 280 °C; IR (ν_{max}): 2994.91 ([sbond]NH), 2904.27 ([sbond]NH), 2823.31 ([sbond]NH), 1719.23 (C[dbond]O), 1670.05 (C[dbond]O), 1646.16 (C[dbond]O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 2.052–2.090 (d, 3H, CH_3, $J = 11.4$ Hz), 5.715 (s, 1H, [sbond]CH), 5.836 (s, 1H, Ar[sbond]H), 6.934–6.961 (d, 2H, Ar[sbond]H, [sbond]NH, $J = 8.1$ Hz), 7.190–7.218 (d, 3H, Ar[sbond]H, $J = 8.4$ Hz), 9.682 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.45, 39.22, 78.14, 78.33, 78.58, 78.80, 79.02, 89.68, 103.39, 103.79, 118.15, 129.17, 130.57, 142.84, 151.91, 159.40, 166.03, 167.31, 170.09 ppm; mass (*m/z*): 402.1988 (M^+). Anal. calcd. For $C_{17}H_{12}BrN_3O_4$: C, 50.77; H, 3.01; N, 10.45%. Found: C, 50.72; H, 3.00; N, 10.42%.

5-(3-Methoxyphenyl)-8-methyl-5,10-dihydro-2H-pyrano-[3′,4′:5,6]pyrido[2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_8$)

White powder; yield 82%; m.p. 278 °C; IR (ν_{max}): 2968.87 ([sbond]NH), 2901.38 ([sbond]NH), 2813.63 ([sbond]NH), 1705.73 (C[dbond]O), 1661037 (C[dbond]O), 1635.34 (C[dbond]O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 2.101 (s, 3H, CH_3), 3.643 (s, 3H, [sbond]OCH_3), 5.702 (s, 1H, [sbond]NH), 5.868 (s, 1H, [sbond]CH), 6.526–6.676 (m, 4H, Ar[sbond]H), 6.983–7.009 (d, 2H, Ar[sbond]H, $J = 7.8$ Hz), 10.038 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.41, 33.49, 55.10, 89.68, 91.50, 103.52, 103.74, 109.47, 113.63, 119.67, 128.77, 145.78, 151.20, 159.18, 159.32, 165.95, 166.95, 170.01 ppm; mass (*m/z*): 353.3288 (M^+). Anal. calcd. For $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89%. Found: C, 61.16; H, 4.24; N, 11.85%.

5-(4-Methoxyphenyl)-8-methyl-5,10-dihydro-2H-pyrano [3′,4′:5,6] pyrido [2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_9$)

White powder; yield 92%; m.p. 274 °C; IR (ν_{max}): 3009.37 ([sbond]NH), 2826.17 ([sbond]NH), 1717.30 (C[dbond]O), 1670.05 (C[dbond]O), 1634.38 (C[dbond]O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 2.050–2.090 (s, 3H, CH_3), 3.920 (s, 3H, [sbond]OCH_3), 5.702 (s, 1H, [sbond]CH), 5.824 (s, 1H, Ar[sbond]H), 6.619–6.647 (d, 2H, Ar[sbond]H, $J = 8.4$ Hz), 6.922[sbond]6.981 (t, 3H, Ar[sbond]H, [sbond]NH), 7.212 (s, 2H, Ar[sbond]H), 9.791(s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.46, 32.74, 55.17, 90.00, 102.24, 103.90, 104.16, 113.16, 127.90, 134.87, 151.98, 157.07, 158.57, 158.99, 166.20, 167.49, 170.06 ppm; mass (*m/z*): 353.3288 (M^+). Anal. calcd. For $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89%. Found: C, 61.14; H, 4.22; N, 11.86%.

8-Methyl-5-(thiophen-2-yl)-5,10-dihydro-2H-pyrano[3′,4′:5,6]pyrido[2,3-d]pyrimidine-2,4,6(1H,3H)-trione (4d$_{10}$)

White powder; yield 86%; m.p. 254 °C; IR (ν_{max}): 3043.12 ([sbond]NH), 2834.85 ([sbond]NH), 1748.16 (C[dbond]O), 1694.16 (C[dbond]O), 1646.91 (C[dbond]O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 1.831 (s, 3H, CH_3), 6.231 (s, 1H, [sbond]CH), 7.956–7.991 (d, 3H, Ar[sbond]H, [sbond]NH, $J = 10.5$ Hz), 8.536 (s, 2H, Ar[sbond]H), 9.840 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.06, 33.20, 90.08, 98.99, 101.22, 102.73, 103.05, 105.14, 126.07, 128.83, 130.56, 140.06, 141.98, 151.96, 160.11, 161.49, 165.56, 167.96, 170.30, 173.66 ppm; mass (*m/z*): 329.3305 (M^+). Anal. calcd. For $C_{15}H_{11}N_3O_4S$: C, 54.71; H, 3.37; N, 12.76%. Found: C, 54.68; H, 3.34; N, 12.72%.

5-(4H-1-Benzopyran-3-yl)-8-methyl-5,10-dihydro-2H-pyrano[3′,4′:5,6] pyrido [2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_{11}$)

White powder; yield 90%; m.p. >300 °C; IR (ν_{max}): 3020.94 ([sbond]NH), 2871.49 ([sbond]NH), 2817.49 ([sbond]NH), 1725.01 (C[dbond]O), 1663.30 (C[dbond]O), 1626.66 (C[dbond]O), 1604.48 (C[dbond]C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.059 (s, 3H, CH_3), 5.684 (s, 1H, Ar[sbond]H, [sbond]CH), 7.773–8.268 (m, 7H, Ar[sbond]H, [sbond]NH), 9.714 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.44, 33.63, 78.22, 78.65, 79.10, 99.98, 101.21, 118.21, 123.63, 124.95, 125.52, 133.51, 151.45, 151.86, 154.10, 156.11, 165.46, 176.89 ppm; mass (*m/z*): 377.3502 (M^+). Anal. calcd. For $C_{20}H_{15}N_3O_5$: C, 63.66; H, 4.01; N, 11.14%. Found: C, 63.62; H, 3.96; N, 11.10%.

5-(3,4-Difluorophenyl)-8-methyl-5,10-dihydro-2H-pyrano [3′,4′:5,6] pyrido [2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_{12}$)

White powder; yield 80%; m.p. 288 °C; IR (ν_{max}): 3019.98 ([sbond]NH), 2887.88 ([sbond]NH), 2817.49 ([sbond]NH), 1740.44 (C[dbond]O), 1662.34 (C[dbond]O), 1585.20 (C[dbond]C), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.098 (s, 3H, CH_3), 5.148 (s, 1H, [sbond]CH), 5.799–5.855 (d, 2H, Ar[sbond]H, [sbond]NH, $J = 16.8$ Hz), 6.798–6.828 (d, 1H, Ar[sbond]H, $J = 9.0$ Hz), 7.166–7.196 (d, 2H, Ar[sbond]H, $J = 9.0$ Hz), 7.855–7.881 (s, 1H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.37, 19.80, 33.10, 89.32, 103.01, 103.66, 115.37, 115.58, 116.96, 151.16, 151.67, 159.82, 165.83, 166.74, 170.10 ppm; mass (*m/z*):359.2837 (M^+). Anal. calcd. For $C_{17}H_{11}F_2N_3O_4$: C, 56.83; H, 3.09; N, 11.70%. Found: C, 56.80; H, 3.06; N, 11.64%.

5-(2H-1,3-Benzodioxol-5-yl)-8-methyl-5,10-dihydro-2H-pyrano[3′,4′:5,6] pyrido [2,3-d] pyrimidine-2,4,6(1H,3H)-trione (4d$_{13}$)

White powder; yield 92%; m.p. 259 °C; IR (ν_{max}): 2904.27 ([sbond]NH), 2817.49 ([sbond]NH), 1667.16 (C[dbond]O), 1633.41 (C[dbond]O), 1571.70 (C[dbond]C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ, 2.097 (s, 3H, CH_3), 5.695 (s, 1H, [sbond]NH), 5.826 (s, 1H, [sbond]CH), 6.502–6.547 (t, 2H, Ar[sbond]H), 7.149 (s, 3H, Ar[sbond]H), 7.993 (s, 1H, Ar[sbond]H), 9.821 (s, 2H, [sbond]NH) ppm; 13C NMR (75 MHz, DMSO-d_6): δ, 19.34, 33.23, 90.01, 100.83, 102.02, 103.74, 105.22, 107.60, 107.76, 119.58, 137.71, 144.85, 147.25, 151.25, 151.78, 159.59, 165.94, 167.09, 170.05 ppm; mass (*m/z*): 367.3123 (M^+). Anal. calcd. For $C_{18}H_{13}N_3O_6$: C, 58.86; H, 3.57; N, 11.44%. Found: C, 58.82; H, 3.52; N, 11.40%.

Conclusion

To summarize, we have reported the use of glycerol:proline (1:1) LTTM for one-pot multicomponent synthesis of pyrano[3′,4′:5,6]pyrido[2,3-*d*]pyrimidine derivatives. The synthesis of LTTM or

DES is more energy efficient and can be synthesized by simply mixing and heating the components. This LTTM has been environmentally benign solvent and recyclable to facilitate several organic transformations. This protocol is environmentally viable with no chromatographic purification, simple work up, suitable reaction time, good to an excellent yield and admirable correlation green metrics calculations and reusability of LTTM. The present methodology will be attractive and convenient, and green alternative path for simple catalytic organic transformations to develop structurally diverse bioactive molecules.

DPPH radical scavenging activity is a good biological method to check the antioxidant activity of compounds. The molecular docking study and antioxidant activity of lead molecules showed excellent activities as compared to standard method. The recent study will motivate medicinal chemist to find new better antioxidant molecule among the structures.

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