An efficient multicomponent synthesis of 1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione derivatives and evaluation of their α-amylase and α-glucosidase inhibitory activity

Mamata Devendra Naik1, Yadav D Bodke2, Prashantha J3 and Jayanth K Naik4

Abstract
In this paper, we report the synthesis of novel 1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione derivatives 5(a–j) by a facile multicomponent reaction. The structures of all the newly synthesized compounds were characterized by different spectroscopic techniques including infrared, nuclear magnetic resonance (1H and 13C) and mass spectral analysis. All the new compounds were assessed for their in vitro α-amylase and α-glucosidase enzyme inhibitory potential. The results of the assays revealed that all compounds showed different enzyme inhibition activities. The concentration required to inhibit enzyme activity is less in the case of α-glucosidases than for α-amylases, that is, the synthesized compounds are more potent in arresting α-glucosidase enzyme activity.

Keywords
pyrano-pyrimidine, α-glucosidase, α-amylase, multicomponent, diabetes mellitus

Introduction
Organic synthesis is one of the primary methods for the preparation of chemicals with broad pharmaceutical and photophysical properties. Heterocyclic compounds containing one or more functionalized units are of great significance in daily life because of their pronounced medicinal value.1 Molecular hybridization is a new approach in rational drug design and the development of novel molecules or prototypes in which chemical modifications made to existing pharmacophores leads to the new hybrid compounds. These can have reduced toxicity, improved physicochemical properties and biological activities, and most importantly, enhanced specificity towards the particular receptor.2,3

Pyran and its derivatives are six-membered oxygen-containing heterocyclic compounds. Among pyran derivatives, 4H-pyran constitutes a vital structural subunit of biologically active natural products as well as synthetic products. A number of naturally occurring products such as carbohydrates, alkaloids, pheromones and polyether antibiotics have a 4H-pyran core unit.4,5 Fused pyrans and substituted pyrans found in both natural and synthetic compounds constitute an important heteroatomic framework with useful pharmacological activities including antibacterial, antiviral, anticoagulant, anti-anaphylactic,

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1Department of P.G. Studies and Research in Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga, India
2Department of P.G. Studies and Research in Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga, India
3Department of P.G. Studies and Research in Microbiology, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga, India
4Research Associate, ITC Life Science and Technology Centre, Bangalore, India

Corresponding author: Yadav D Bodke, Department of P.G. Studies and Research in Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga 577451, India.
Email: ydbodke@gmail.com
anticaner and diuretic activities and activity towards neurodegenerative disorders.\textsuperscript{6,7}

Pyrimidine and its derivatives are the important class of aza-aromatic scaffolds found in biomolecules such as DNA and RNA. The pyrimidine core skeleton is also found in many living organisms.\textsuperscript{8} The synthesis of pyrimidine and condensed pyrimidine molecules has been the subject of substantial interest because of their usefulness in biological and clinical applications.

Diabetes mellitus (DM), a multifactorial chronic syndrome, is characterized by the insufficient release of insulin from the pancreas which regulates glucose homeostasis.\textsuperscript{9} DM is of three categories, namely, type I, type II and gestational diabetes among which type II contributes to serious medicinal concerns since it is associated with several life-threatening health problems such as metabolic syndrome, cardiovascular disease, foot ulcers, renal function recession and blindness.\textsuperscript{10,11} One of the most important therapeutic methods to prevent raising chronic hyperglycaemia is to control the glucose level in blood by inhibiting the activity of digestive enzymes which catalyse the hydrolysis of carbohydrates.\textsuperscript{12}

Pyrido[2,3-d]pyrimidines, pyrido[2,3-d]pyrimidin-4(5H)-ones, pyrido[4,5-d]pyrimidines or pyrimido[4,5-d]pyrimidin-4(5H)-ones are uracil derivatives and play a distinct and significant role in the field of bio-organic chemistry. Pyrano-pyrimidine derivatives are characterized by the fusion of pyran and pyrimidine rings and the resulting pharmacophores are found to possess abundant biological activities. They form a substantial group of pharmacologically active heterocycles and have been given considerable attention by synthetic and medicinal chemists.\textsuperscript{13,14} In this study, we have synthesized some 1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione derivatives by three-component reactions involving 4-hydroxycoumarin, barbituric acid and piperidine (Scheme 1). The synthesized molecules were screened for hyperglycaemic activity.

Result and discussion

Chemistry

Fused polyheterocycles are invaluable structural motifs in the field of organic chemistry and find application in pharmaceutical and material science. Molecular hybridization facilitates the formation of these fused hybrids and involves the fusion of different pharmacophores in a single framework. The structural modifications stem from the fusion of two or more bioactive scaffolds that impart complexity and more pronounced biological activities to the resulting polycyclic system than that of parent individual heterocycles.

As an initial trial reaction, we performed a multicomponent reaction involving 4-hydroxycoumarin (1 mmol), barbituric acid (1 mmol), piperidine (1 mmol) and benzaldehyde (1 mmol) as a model reaction. The reaction was first carried out by grinding all the substrates in the absence of solvent but the result obtained was not encouraging. The formation of the product was negligible even after prolonged grinding (Table 1, Entry 1). Subsequently, the reaction was carried out using different solvents such as Dichloromethane (DCM), ethyl acetate, acetonitrile, ethanol, methanol, water and a mixture of solvents. When the reaction was carried out using DCM, ethyl acetate, water, acetonitrile, under both reflux and room temperatures, little solid product was formed and thin layer chromatography (TLC) showed the presence of mixtures (Table 1, Entries 2–5).

In an endeavour to improve the yield of the product, the same reaction was repeated in ethanol and methanol. Surprisingly, an 89% yield of the product was obtained in methanol at room temperature. This indicated that the nature of the solvent had significant effect on the reaction rate and yield of the product. Among the solvents tested, methanol was found to be the best (Table 1, Entries 6–9). Furthermore, an increase in the temperature did not show any significant improvement in the product yield and reaction rate.

Using this multicomponent reaction, equimolar mixtures of 4-hydroxycoumarin, barbituric acid, piperidine and a substituted aldehyde were used for the construction of coumarin-based pyrano-pyrimidine derivatives. Figure 1 shows a plausible mechanistic pathway for the formation of these novel 1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-diones. This may involve a Knoevenagel condensation of the aldehyde and barbituric acid in the presence of piperidine followed by the nucleophilic attack of the 4-hydroxycoumarin with dehydration followed by ring-opening of coumarin nucleus by piperidine leading to the target compound.

The structures of all the newly synthesized compounds were characterized by spectroscopic techniques. The Fourier transform infrared (FTIR) spectrum of compound 5b showed an absorption band at 3622.43 cm\textsuperscript{-1} which was attributed to –OH stretching. Two other absorption bands

![Scheme 1. The synthetic strategy used to get the title compounds 5(a–j).](image)

| Entry | Solvent | Temperature (°C) | Time (h) | Yield (%) |
|-------|---------|----------------|----------|-----------|
| 1     | Solvent free | –           | 8        | trace     |
| 2     | DCM     | 40            | 8        | 30        |
| 3     | Ethyl acetate | 70        | 6        | 45        |
| 4     | Water   | 85            | 12       | 40        |
| 5     | CH\(_3\)CN | 75           | 6        | 35        |
| 6     | MeOH    | –             | 4        | 89        |
| 7     | EtOH    | –             | 4        | 80        |
| 8     | EtOH    | 70            | 6        | 80        |
| 9     | MeOH    | 60            | 6        | 88        |
| 10    | EtOH + Water (1:1) | 75 | 6 | 52 |
| 11    | MeOH + Water (1:1) | 75 | 6 | 55 |

Table 1. Optimization of reaction condition.
were observed at 1722.65 and 1658.09 cm\(^{-1}\) corresponding to the carbonyl groups of the pyrimidine ring and a piperidine carbonyl group. The absorption band at 3351.03 cm\(^{-1}\) corresponds to the –NH stretching frequency of the pyrimidine moiety. The \(^1\)H NMR spectrum of compound 5b displayed three signals at δ 1.51, 1.59 and 2.97 ppm corresponding to the –CH\(_2–\) protons of the piperidine moiety. The appearance of a singlet at 6.06 ppm signifies the formation of the product. The OH proton derived from the coumarin appears at 16.93 ppm. Furthermore, the \(^13\)C NMR spectrum of compound 5b is in good agreement with the structure assigned to this compound. The mass analysis of compound 5b displayed a molecular ion peak with an m/z value of 479 (M), which is consistent with the molecular formula C\(_{25}\)H\(_{22}\)ClN\(_3\)O\(_5\). The physicochemical data of all the compounds with electron-donating and electron-withdrawing groups (CH\(_3\) and OCH\(_3\)) showed a remarkable decline in the enzyme inhibitory activity (high IC\(_50\) value). Compound 5a with no substitution on aromatic ring showed moderate activity.

The difference in the activity profile shown by the compounds with the same functional group on the aromatic ring may be due to the position of substituents. If we compare compounds 5g and 5h, both contain the hydroxyl group on the para position but showed a difference in the activity which may be attributed due to naphthol ring in compound 5h which presented high activity. Compound 5i with the hydroxyl group on para position showed less efficacy due to the presence of methoxy group, which may reduce the activity of the molecule towards the enzyme inhibition (which can be justified by the compound 5f with least activity).

In vitro \(\alpha\)-amylase inhibitory activity

The \(\alpha\)-amylase inhibitory potencies of the synthesized compounds 5(a–j) were assessed by determining the concentration required for the 50% inhibition (IC\(_{50}\)) of enzyme activity and comparing this with the standard clinically used anti-diabetic drug, acarbose. The assay was performed at different concentrations to decide the IC\(_{50}\); the lower IC\(_{50}\) indicates the stronger enzyme inhibition. The results are presented in Table 3. Among the series, compound 5h showed good inhibition activity with least IC\(_{50}\) (6.490 mM) when compared with standard, acarbose (IC\(_{50}\)=0.2137 mM), followed by compound 5b that had an IC\(_{50}\) value of 9.897 mM. Compounds 5b, 5e and 5g showed moderate activity with the inhibition concentration ranging from 9.897 to 20.131 mM. The rest of the compounds (5e, 5i and 5j) requires high concentrations to arrest the enzyme inhibition.

In vitro \(\alpha\)-glucosidase inhibitory activity

The \(\alpha\)-glucosidase assay was performed to study the inhibition efficiency of the synthesized molecules on the \(\alpha\)-glucosidase enzyme. The assay is based on the mechanism of hydrolysis of PNPG (\(p\)-nitrophenyl-\(\alpha\)-glucopyranoside) by the enzyme to simple sugars.

\[
\text{PNPG} \xrightarrow{\beta\text{-glucosidase}} \text{D-glucose} + \text{p-nitro phenol (yellow color)}
\]
Table 2. Physicochemical data of synthesized compounds 5(a-j).

| Compound | R         | Product     | MF/M. wt      | Yield (%) | m.p. (°C) |
|----------|-----------|-------------|---------------|-----------|-----------|
| 5a       | H         |             | C_{25}H_{23}N_{3}O_{5}/445.43 | 80        | 235–236   |
| 5b       | Cl        |             | C_{25}H_{22}ClN_{3}O_{5}/479.91 | 89        | 232–234   |
| 5c       | Br        |             | C_{25}H_{22}BrN_{3}O_{5}/524.36 | 88        | 236–238   |
| 5d       | F         |             | C_{25}H_{22}FN_{3}O_{5}/436.45 | 87        | 228–230   |
| 5e       | CH\textsubscript{3} | | C_{26}H_{25}N_{3}O_{5}/459.49 | 82        | 234–236   |
| 5f       | OCH\textsubscript{3} | | C_{26}H_{25}N_{3}O_{5}/475.49 | 85        | 236–237   |
| 5g       | OH        |             | C_{25}H_{23}N_{3}O_{6}/461.46 | 78        | 234–235   |
| 5h       | C\textsubscript{6}H\textsubscript{4}(OH) | | C_{29}H_{25}N_{3}O_{6}/511.52 | 70        | 242–244   |
| 5i       | (OCH\textsubscript{3})OH | | C_{26}H_{25}N_{3}O_{6}/491.49 | 74        | 230–231   |
| 5j       | OH        |             | C_{25}H_{23}N_{3}O_{6}/461.46 | 78        | 240–241   |
Concentration (mg mL⁻¹) IC₅₀ (mM)

| Compound | Concentration (mg mL⁻¹) | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 |
|----------|------------------------|----|----|---|-----|------|------|
| 5a       | 69.17 ± 2.89           | 45.79 ± 0.86 | 38.61 ± 1.81 | 18.66 ± 2.21 | 5.42 ± 1.78 | 4.47 ± 0.37 | 22.068 |
| 5b       | 73.37 ± 1.76           | 70.24 ± 1.19 | 51.02 ± 1.76 | 40.00 ± 1.47 | 17.23 ± 0.37 | 14.20 ± 0.97 | 9.897 |
| 5c       | 72.39 ± 2.30           | 52.75 ± 1.88 | 37.61 ± 1.39 | 30.78 ± 0.99 | 25.60 ± 1.12 | 18.78 ± 0.86 | 14.112 |
| 5d       | 72.69 ± 1.56           | 56.92 ± 2.75 | 47.39 ± 2.02 | 22.08 ± 1.63 | 16.21 ± 2.14 | 7.67 ± 1.89 | 16.107 |
| 5e       | 67.20 ± 2.30           | 46.82 ± 2.19 | 23.62 ± 1.64 | 17.59 ± 1.75 | 20.93 ± 1.87 | 6.91 ± 1.15 | 24.723 |
| 5f       | 51.21 ± 1.85           | 39.58 ± 0.63 | 28.86 ± 1.00 | 20.69 ± 1.45 | 21.85 ± 1.56 | 10.90 ± 0.83 | 42.797 |
| 5g       | 66.52 ± 2.34           | 48.97 ± 2.14 | 40.88 ± 0.67 | 22.26 ± 2.11 | 14.93 ± 3.08 | 11.84 ± 2.34 | 20.131 |
| 5h       | 84.85 ± 1.89           | 75.92 ± 2.32 | 63.73 ± 1.84 | 50.56 ± 1.45 | 17.89 ± 1.55 | 6.22 ± 1.05 | 6.490 |
| 5i       | 63.10 ± 2.62           | 48.78 ± 2.07 | 31.10 ± 1.69 | 21.47 ± 2.35 | 13.79 ± 2.28 | 4.59 ± 1.45 | 22.604 |
| 5j       | 59.09 ± 1.99           | 45.53 ± 1.52 | 32.10 ± 2.04 | 21.59 ± 2.33 | 9.6 ± 1.54  | 5.37 ± 0.96 | 27.087 |

Table 3. Relative α-amylase enzyme activity values (%) at different concentrations and IC₅₀ values.

Acarbose is standard inhibitor for α-amylase and the IC₅₀ = 0.2137 mM (at five different concentrations).

The increase in the intensity of the yellow colour developed after the reaction indicated the extent of hydrolysis of the complex sugar to the simple sugars. The inhibitory concentration of the title compounds 5(a–j) was also determined by comparing the IC₅₀ with the standard. From the result, we observed that most of the compounds showed promising activity compared with acarbose (IC₅₀ = 0.3268 mM). As reported earlier, the structural and electronic factors influence the activity profile. Compound 5j with the hydroxyl group on the para position of the aromatic ring showed the highest activity among the series with IC₅₀ value 3.553 mM followed by 5b with chloro substitution on the phenyl ring. Other compounds showed the inhibitory concentration ranging from 5.223 to 6.855 mM. The least activity was shown by the compound without any substitution with IC₅₀ 7.745 mM.

Overall, we observed that the synthesized targets are more capable of inhibiting the activity of α-glucosidase than the α-amylase enzyme. The hydroxyl substituted analogues showed potent activity in both assays; this may be due to the interaction of phenols with the protein and leading to the inhibition of enzyme activity. Tables 3 and 4 and Figures 2 and 3 showed the in vitro α-amylase and α-glucosidase inhibitory activity of compounds 5(a–j).

Conclusion

We have synthesized 10 pyrano-pyrimidine derivatives using multicomponent reaction via a Knoevenagel condensation. The biological evolution of these compounds revealed that all the compounds are active against both digestive enzymes (α-amylase and α-glucosidase). Particularly, the compounds with hydroxyl substitution on the aromatic ring showed better potential with the least IC₅₀ value when compared to other electron-donating groups. On the contrary, the substitution pattern on the phenyl ring also decides the inhibition efficacy of the synthesized targets. Overall, by comparing the IC₅₀ values from both the assays, it was concluded that the synthesized compounds are most potent in inhibiting α-glucosidase enzyme activity than α-amylase.

Experimental section

Materials and methods

The enzymes α-glucosidase from Saccharomyces cerevisiae (Kₐ = 0.5–2.7 μM), Porcine pancreatic α-amylase (Type VI-B, Kₐ = 0.000624–3.05 μM), p-nitrophenyl-α-D-glucopyranoside and other chemicals required for the synthesis were procured from commercial suppliers Sigma-Aldrich and Himedia and used without purification. The completion of the reaction was checked by TLC on silica gel pre-coated aluminium sheets and the chromatograms developed were identified by exposure to UV lamp. The melting point was recorded using an electrothermal apparatus and is uncorrected. Infrared spectra (ν, cm⁻¹) were recorded on FT-IR Bruker Spectrophotometer using KBr pellets, NMR spectra were recorded on Bruker 400-MHz (¹H NMR) and 100-MHz (¹³C NMR) spectrometer using DMSO-d₆ as solvent. The chemical shifts are reported in ppm (δ) with reference to tetramethyl silane as an internal standard and the coupling constants (J) values are expressed in Hertz (Hz). Finally, the mass spectra were recorded using a C-18 column on Shimadzu liquid chromatography-mass spectrometer (LCMS 2010A), Japan.

General procedure for the synthesis of 1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione derivatives 5(a–j)

An equimolar mixture of 4-hydroxy coumarin (1 mmol), barbituric acid (1 mmol), piperidine (1 mmol) and the substituted aldehyde (1 mmol) in methanol (10 mL) was charged in 100-mL round-bottomed flask and the resulting homogeneous mixture was magnetically stirred at room temperature for about 2–4 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the precipitated product was collected by filtration, washed thoroughly with methanol and then with water and finally dried to afford pure product 5(a–j).

7-(2-Hydroxyphenyl)-5-phenyl-6-(piperidine-1-carbonyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (5a): Yield 80%, white powder, m.p.: 235–236°C. IR
Table 4. Relative α-glucosidase enzyme activity values (%) at different concentrations and IC50 values.

| Compound | Concentration (mg mL⁻¹) | IC50 (mM) |
|----------|-------------------------|-----------|
|          | 20                      | 10        | 5         | 2.5        | 1.25       |
| 5a       | 83.30 ± 1.34            | 74.66 ± 1.35 | 58.05 ± 2.08 | 41.56 ± 1.97 | 27.61 ± 1.57 | 7.745 |
| 5b       | 77.30 ± 2.61            | 75.65 ± 2.65 | 68.57 ± 1.69 | 62.44 ± 1.89 | 35.15 ± 2.91 | 4.021 |
| 5c       | 84.40 ± 1.54            | 78.99 ± 2.09 | 60.51 ± 2.61 | 50.90 ± 1.82 | 31.53 ± 2.33 | 5.187 |
| 5d       | 85.91 ± 0.99            | 85.51 ± 0.31 | 76.19 ± 1.46 | 51.40 ± 2.30 | 31.78 ± 2.48 | 5.223 |
| 5e       | 86.52 ± 1.26            | 78.92 ± 2.09 | 68.49 ± 3.07 | 42.09 ± 3.27 | 22.50 ± 2.81 | 6.855 |
| 5f       | 86.83 ± 1.82            | 82.30 ± 1.04 | 70.51 ± 0.83 | 54.51 ± 1.41 | 40.30 ± 1.25 | 4.101 |
| 5g       | 84.53 ± 1.96            | 81.53 ± 2.25 | 55.64 ± 2.96 | 50.88 ± 2.76 | 30.84 ± 1.27 | 6.197 |
| 5h       | 85.33 ± 0.88            | 83.56 ± 2.08 | 67.28 ± 1.83 | 49.78 ± 1.01 | 26.05 ± 0.96 | 5.337 |
| 5i       | 82.59 ± 0.83            | 83.72 ± 1.16 | 74.33 ± 1.59 | 48.77 ± 1.32 | 33.42 ± 1.29 | 4.862 |
| 5j       | 84.47 ± 0.81            | 86.38 ± 1.12 | 72.51 ± 1.56 | 61.62 ± 0.71 | 42.40 ± 1.21 | 3.553 |

Acetobacter is standard inhibitor for α-glucosidase and the IC50 = 0.3268 mM (at five different concentrations).
Figure 2. In vitro α-amylase inhibitory activity of the synthesized compounds 5(a–j).

2H, J = 8.2 Hz, ArH), 7.23 (d, 2H, J = 8 Hz, ArH), 7.51 (t, 2H, J = 8.8 Hz, ArH), 7.75 (d, 1H, J = 7.6 Hz, ArH), 8.18 (s, 1H, ArH), 9.87 (s, 2H, pyrimidine-NH), 16.98 (s, 1H, Ar-OH). $^1$C NMR (100 MHz, DMSO-d$_6$): $\delta$ = 22.6, 33.6, 44.2, 55.5, 89.3, 106.1, 115.6, 119.8, 122.8, 124.7, 127.3, 128.5, 131.3, 133.4, 140.8, 152.4, 152.6, 164.7. LCMS: m/z = 475 (M). Anal. calcd for C$_{26}$H$_{25}$N$_3$O$_6$: C, 65.67; H, 5.30; N, 8.84; found: C, 65.52; H, 5.27; N, 8.67.

5,7-Bis(2-hydroxyphenyl)-6-(piperidine-1-carbonyl)-1H-pyrano[2,3-d]pyrimidine-2,4(3H,5H)-dione (5g): Yield 78%, white powder, m.p.: 234–235°C. IR (KBr, v cm$^{-1}$): 3646.73, 3356.43, 1777.42, 1680.57. $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ = 1.52 (quintet, 2H, J = 4.8 Hz, piperidine), 1.62 (quintet, 4H, J = 4.8 Hz, piperidine), 3.02 (t, J = 4.8 Hz, piperidine), 4.86 (s, 1H, Ar-OH), 6.59 (s, 1H, ArCH$_2$), 6.96 (m, 2H, ArH), 7.15 (d, J = 8 Hz, 2H, ArH), 7.26 (t, J = 8.6 Hz, 1H, ArH), 7.49 (1H, d, J = 8.4 Hz, ArH), 7.75 (s, 1H, ArH), 8.19 (s, 1H, ArH), 10.02 (s, 2H, pyrimidine-NH), 17.12 (s, 1H, Ar-OH). $^1$C NMR (100 MHz, DMSO-d$_6$): $\delta$ = 22.6, 32.9, 44.2, 55.3, 92.1, 105.2, 117.8, 120.5, 122.98, 124.4, 127.2, 128.5, 131.3, 133.4, 140.8, 152.4, 152.6, 164.7. LCMS: m/z = 462 (M+1). Anal. calcd for C$_{25}$H$_{23}$N$_3$O$_6$: C, 65.07; H, 5.02; N, 9.11; found: C, 64.89; H, 4.87; N, 8.88.

5-(3-Hydroxynaphthalen-2-yl)-7-(2-hydroxyphenyl)-6-(piperidine-1-carbonyl)-1H-pyran[2,3-d]pyrimidine-2,4(3H,5H)-dione (5h): Yield 70%, white powder, m.p.: 242–244°C. IR (KBr, v cm$^{-1}$): 3626.34, 3486.74, 2876.47, 1785.47, 1684.64. $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ = 1.53 (quintet, 2H, J = 4.6 Hz, piperidine), 1.62 (quintet, 4H, J = 4.8 Hz, piperidine), 4.26 (s, 1H, Ar-OH), 6.06 (s, 1H, ArCH$_2$), 6.94 (m, 2H, ArH), 7.15 (d, J = 8 Hz, 2H, ArH), 7.26 (t, J = 8.6 Hz, 1H, ArH), 7.49 (1H, d, J = 8.4 Hz, ArH), 7.75 (s, 1H, ArH), 8.19 (s, 1H, ArH), 10.02 (s, 2H, pyrimidine-NH), 17.12 (s, 1H, Ar-OH). $^1$C NMR (100 MHz, DMSO-d$_6$): $\delta$ = 22.0,
22.4, 27.1, 28.2, 34.9, 44.2, 89.6, 105.6, 116.0, 116.6, 118.9, 122.4, 123.2, 123.8, 124.3, 125.3, 125.0, 125.9, 126.55, 127.9, 132.4, 131.3, 132.8, 133.1, 141.5, 151.9, 152.5, 152.9, 154.1, 163.9, 164.2, 165.8, 168.1. LCMS: \( m/z = 512 \) (M). Anal. calcd for C\(_{29}\)H\(_{25}\)N\(_3\)O\(_6\): C, 68.09; H, 4.93; N, 8.21; found: C, 67.82; H, 4.86; N, 8.12.

5-(4-Hydroxy-3-methoxyphenyl)-7-(2-hydroxyphenyl)-6-(piperidine-1-carbonyl)-1\( \text{H} \)-pyrano[2,3-d]pyrimidine-2,4(3\( \text{H},5\text{H} \))-dione (5i): Yield 74%, white powder, m.p.: 230–231 °C. IR (KBr, \( \nu \text{cm}^{-1} \)): 3687.64, 3456.86, 2875.64, 1796.62, 1686.74. \(^1\text{H} \) NMR (400 MHz, DMSO-\( \text{d}_6 \)): \( \delta = 1.52 \) (quintet, 2H, \( J = 4.8 \text{ Hz} \), piperidine), 1.59 (quintet, 4H, \( J = 4.6 \text{ Hz} \), piperidine), 3.23 (t, 4H, \( J = 4.8 \text{ Hz} \), piperidine), 3.56 (s, 3H, -OCH\(_3\)), 4.86 (s, 1H, Ar-OH), 6.08 (s, 1H, Ar-CH\(_3\)), 7.14 (d, 2H, \( J = 8 \text{ Hz} \), ArH), 7.26 (d, 2H, \( J = 8.2 \text{ Hz} \), ArH), 7.30 (t, 1H, \( J = 7.6 \text{ Hz} \), ArH), 7.48 (d, 1H, \( J = 8.2 \text{ Hz} \), ArH), 7.84 (s, 1H, ArH), 10.24 (s, 2H, pyrimidine-NH), 17.18 (s, 1H, Ar-OH). \(^{13}\text{C} \) NMR (100 MHz, DMSO-\( \text{d}_6 \)): \( \delta = 21.9, 22.7, 31.2, 44.2, 51.2, 55.3, 55.88, 87.8, 104.9, 106.3, 118.7, 121.6, 122.1, 122.9, 123.4, 127.6, 130.5, 152.8, 155.3, 160.7, 164.44, 167.8. LCMS: \( m/z = 512 \) (M+1). Anal. calcd for C\(_{27}\)H\(_{27}\)N\(_3\)O\(_7\): C, 63.54; H, 5.13; N, 8.55; found: C, 63.27; H, 5.02; N, 22.54.

7-(2-Hydroxyphenyl)-5-(4-hydroxyphenyl)-6-(piperidine-1-carbonyl)-1\( \text{H} \)-pyrano[2,3-d]pyrimidine-2,4(3\( \text{H},5\text{H} \))-dione (5j): Yield 78%, white powder, m.p.: 240–241 °C. IR (KBr, \( \nu \text{cm}^{-1} \)): 3645.42, 3478.84, 1675.89, 1796.48. \(^1\text{H} \) NMR (400 MHz, DMSO-\( \text{d}_6 \)): \( \delta = 1.52 \) (quintet, 2H, \( J = 4.8 \text{ Hz} \), piperidine), 1.62 (quintet, 4H, \( J = 4.8 \text{ Hz} \), piperidine), 2.98 (t, 4H, \( J = 4.8 \text{ Hz} \), piperidine), 4.88 (s, 1H, ArH), 7.14 (d, 2H, \( J = 8 \text{ Hz} \), ArH), 7.26 (d, 2H, \( J = 8.2 \text{ Hz} \), ArH), 7.30 (t, 1H, \( J = 7.6 \text{ Hz} \), ArH), 7.48 (d, 1H, \( J = 8.2 \text{ Hz} \), ArH), 7.84 (s, 1H, ArH), 10.24 (s, 2H, pyrimidine-NH), 17.18 (s, 1H, Ar-OH). \(^{13}\text{C} \) NMR (100 MHz, DMSO-\( \text{d}_6 \)): \( \delta = 21.9, 22.7, 31.2, 44.2, 51.2, 55.3, 55.88, 87.8, 104.9, 106.3, 118.7, 121.6, 122.1, 122.9, 123.4, 127.6, 130.5, 152.8, 155.3, 160.7, 164.44, 167.8. LCMS: \( m/z = 512 \) (M+1). Anal. calcd for C\(_{27}\)H\(_{27}\)N\(_3\)O\(_7\): C, 63.54; H, 5.13; N, 8.55; found: C, 63.27; H, 5.02; N, 22.54.

**Figure 3.** In vitro \( \alpha \)-glucosidase inhibitory activity of the synthesized compounds 5(a–j). In Figure 3, compounds (1–10) represent 5(a–j).
hydroxy), 5.98 (s, 1H, -ArCH-), 7.04 (d, 2H, J=8 Hz, ArH), 7.18 (d, 2H, J=8.2 Hz, ArH), 7.27 (t, 2H, J=7.2 Hz, ArH), 7.46 (s, 1H, ArH), 8.05 (s, 1H, ArH), 9.96 (s, 2H, pyrimidine-NH), 16.78 (s, 1H, Ar-OH). 13C NMR (100 MHz, DMSO-d6): δ = 22.5, 32.8, 44.2, 55.3, 92.2, 105.2, 117.8, 120.5, 121.9, 124.5, 126.6, 128.5, 130.3, 133.6, 143.2, 152.8, 157.6, 163.8, 167.5. LC-MS: m/z = 461 (M). Anal. calcd for C_{25}H_{23}N_{3}O_{6}: C, 65.07; H, 5.02; N, 9.11; found: C, 64.88; H, 4.86; N, 8.89.

**Biological studies**

**In vitro α-amylase inhibitory assay.** α-Amylase inhibitory activity was established in accordance with the earlier reported method with slight modification.15 The test samples were prepared by dissolving synthesized compounds at different concentrations (20, 10, 5, 2.5, and 0.625 mg mL⁻¹) in DMSO. A volume of 40 µL of sample and 40 µL of the α-amylase solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was incubated at 25°C for 10 min. After pre-incubation, 40 µL 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added as substrate. The resultant reaction mixture was incubated at 25°C for 10 min, then dinitrosalicylic acid as colour reagent was added and the test tubes were incubated in a boiling water bath for about 5 min and then cooled at room temperature. The reaction mixture was diluted by adding 900 µL of distilled water and the contents were mixed properly. 50 µL of reaction mixture was taken from the test tube and loaded to 96-well microplate and the absorbance was measured at 405 nm using multiskan sky (Thermo Scientific, USA). Acarbose was used as a reference standard and all experiments were carried out in triplicates. Percent inhibition was calculated using the following equation

\[
\text{Inhibition (\%) = } \left( \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Test} / \text{Abs of Control}} \right) \times 100
\]

**In vitro α-glucosidase inhibitory assay.** The α-glucosidase inhibition activity of the synthesized compounds was determined using the assay described by Sancheti et al., with slight modification.16 Different concentrations (20, 10, 5, 2.5, and 1.25 mg mL⁻¹) synthesized compounds were prepared by dissolving in DMSO. An aliquot of 20 µL of the test sample was added to the 50 µg mL⁻¹ α-glucosidase solution followed by the addition of 60 µL in phosphate buffer solution (pH 6.8) into the 96-well plate. The contents were mixed and incubated for 5 min and then 10 µL of 10 mM ρ-nitrophenyl- α-D-glucoside solution (PNP-GLUC) was added as a substrate and further incubated at 37°C for 20 min. Then, 25 µL of Na₂CO₃ solution (100 mM) was added and the absorbance was measured at 405 nm using multiskan sky (Thermo Scientific). All experiments were carried out in triplicates. Percent inhibition was calculated using the following equation

\[
\text{Inhibition (\%) = } \left( \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Test} / \text{Abs of Control}} \right) \times 100
\]

**Statistical analysis**

The experiments were performed in triplicates and the results of the bioassays were expressed as mean ± SE and the IC₅₀ value was obtained by nonlinear regression analysis using GraphPad Prism software.

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**ORCID iD**

Yadav D Bodke https://orcid.org/0000-0003-2851-3492

**Supplemental material**

Supplemental material for this article is available online.

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