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Short communication

Synthesis of some 5-phenylisoxazole-3-carboxylic acid derivatives as potent xanthine oxidase inhibitors

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ABSTRACT

A number of 5-phenylisoxazole-3-carboxylic acid derivatives (**5a**–**e**, **11a**–**e**) were synthesized and analyzed for their ability to inhibit xanthine oxidase. Most of the compounds exhibited potency levels in the micromolar/submicromolar range. The presence of a cyano group at the 3-position of phenyl moiety turned out to be the preferred substitution pattern, as its transformation into the nitro group determined a general reduction of the inhibitory potency. A molecular modeling study on compound **11a** was performed to gain an insight into its binding mode with xanthine oxidase, and to provide the basis for further structure-guided design of new non-purine xanthine oxidase inhibitors related with 5-phenylisoxazole-3-carboxylic acid scaffold.

1. Introduction

Gout and hyperuricemia, associated with an elevated uric acid (UA) level in the blood, are common human metabolic disorders. These lead to the accumulation of UA in joints and kidneys, which in turn causes gouty arthritis and UA nephrolithiasis [1]. Xanthine oxidase (XO) is a key enzyme in the last two steps of purine metabolic pathway, catalyzing the oxidation of hypoxanthine to xanthine, and then to UA [2]. Since reducing plasma levels of UA can prevent gout, therapy is possible with XO inhibitors that block the production of UA from purine [3]. Allopurinol (Fig. 1), a known XO inhibitor and an analogue of hypoxanthine, has been widely prescribed as a treatment for hyperuricemia and gout. In some cases, however, severe life-threatening side effects have been reported. These include a toxicity syndrome dramatized by eosinophilia, vasculitis, rash hepatitis, and progressive renal failure [4]. Therefore, novel non-purine alternatives to allopurinol with potent XO inhibitory activity, but possessing fewer side effects are in great demand.

Under efforts to find novel XO inhibitors without purine backbone, 2-phenylthiazoles [5] and 1-phenylpyrazoles [6] had been designed and tested as xanthine oxidase inhibitors. Among them, febuxostat and Y-700 (Fig. 1) were shown to be promising xanthine oxidase inhibitors [5,6]. The former had already been approved in Europe last year and in the US this year [7]. Encouraged by the outstanding potency of these inhibitors, we attempt to adopt other five-membered rings, such as isosteres of pyrazole and thiazole, to design their analogues with the hope of extending the structure–activity relationships of non-purine XO inhibitors and finding more potent compounds.

In this paper, we describe the synthesis and preliminary structure–activity relationships of 5-phenylisoxazole-3-carboxylic acid derivatives as potent XO inhibitors.

2. Results and discussion

2.1. Chemistry

The synthesis of the target 5-phenylisoxazole-3-carboxylic acid derivatives **5a**–**e** and **11a**–**e** was performed as outlined in Scheme 1.

The commercially available 1-(4-hydroxyphenyl)ethanone was nitrated with concentrated nitric acid in hot acetic acid to give 1-(4-hydroxy-3-nitrophenyl)ethanone **1**, which was then alkylated with appropriate alkyl halide in DMF in the presence of potassium carbonate to obtain 1-(4-alkyloxy-3-nitrophenyl)ethanones **2a**–**e** [8] (Scheme 1).

The 1-(4-alkyloxy-3-cyanophenyl)ethanones **8a**–**e**, as the key intermediates for the synthesis of target compounds **11a**–**e**, were prepared through a three-step sequence. In the process, 1-(4-hydroxyphenyl)ethanone was treated with iodine and potassium iodide in aqueous ammonia hydroxide to afford 1-(4-hydroxy-3-

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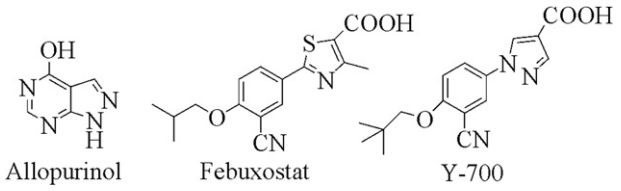


Fig. 1. Chemical structures of allopurinol, febuxostat, and Y-700.

iodophenyl)ethanone **6** [9], which was alkylated as described for **2b** [8] and subsequently cyanided with cuprous cyanide in DMF [10] (Scheme 1).

The diketo esters **3a–e** and **9a–e**, which exclusively exist in their enol tautomeric forms, were obtained by the condensation of **2a–e** and **8a–e** with diethyl oxalate in THF in the presence of sodium ethoxide [11,12]. Finally, cyclocondensation of the appropriate diketo ester with hydroxylamine hydrochloride in refluxing ethanol provided isoxazoles **4a–e** and **10a–e** [13], which were then hydrolyzed with sodium hydroxide to obtain (after acidification) the target 5-phenylisoxazole-3-carboxylic acid derivatives **5a–e** and **11a–e** [14].

The structures of the synthesized target compounds were elucidated by TOF-HRMS, ^{1}H NMR, and IR findings. All spectral data were in accordance with assumed structures. In the TOF-HRMS analysis, compound **11a** showed M + H ion peak, while the other compounds showed M + Na ion peaks. The IR spectra displayed the nitro group stretching vibrations at 1531–1533 cm^{-1} for compounds **5a–e**, and the cyano group stretching vibrations at 2223–2229 cm^{-1} for compounds **11a–e**. In the ^{1}H NMR spectra, the H-4 of isoxazole was observed around 7.50 ppm as a singlet. In the case of compounds **5b**, **5d**, **5e**, **11a**, and **11b**, it was overlapped with the protons of benzene rings.

2.2. Biological activity

The in vitro bovine XO inhibitory activity of compounds **5a–e** and **11a–e** was measured spectrophotometrically by following uric acid levels at 295 nm [15,16]. Febuxostat was included as a reference compound. The results are shown in Tables 1 and 2. In general, most of the 5-phenylisoxazole-3-carboxylic acid derivatives exhibited potent inhibitory activity, but much lower than that of febuxostat. Although the number of synthesized target compounds is limited, some interesting characteristics for the XO inhibitory potency of 5-phenylisoxazole-3-carboxylic acid derivatives could be inferred.

The presence of a cyano group at the 3-position of phenyl moiety (**11a–e**) caused a significant improvement in the activity with respect to the nitro group (**5a–e**). In particular, compounds **11a**, **11b**, and **11d** showed submicromolar inhibitory activity. The same effect also happened in XO inhibitors 1-phenypyrazoles, represented by Y-700 [6].

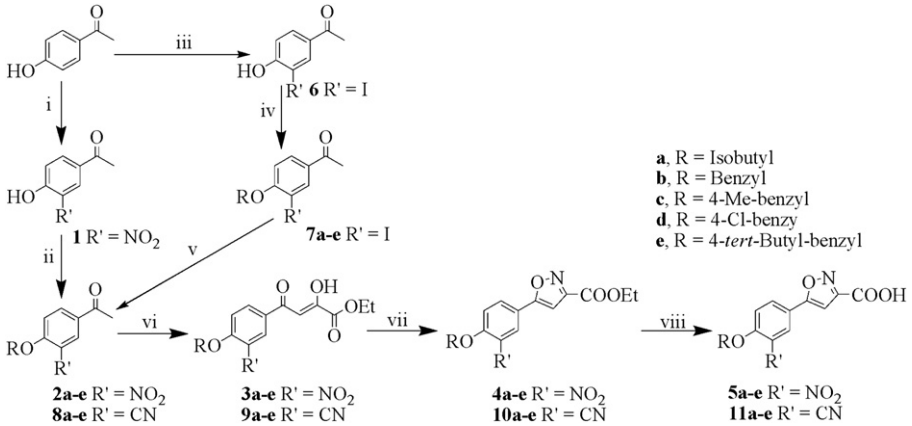
The different alkyloxy groups at the 4-position of phenyl moiety were introduced to verify the influence of substitution pattern on the inhibitory potency of these molecules. Within the series, compounds **5a** and **11a** bearing isobutyl substituent proved to be extremely potent, with an IC_{50} value of 1.00 and 0.36 μM, respectively. The replacement of isobutyl group with benzyl group decreased the inhibitory effectiveness, similar to the case of compounds **5b** and **11b**. In order to explore the possibility of improving the inhibitory activity of benzyl-substituted derivatives, compounds **5c–e** and **11c–e** were synthesized. As shown in Tables 1 and 2, except for compounds **5c** and **11c** that proved to be inactive, most of the compounds exhibited potency levels in the micromolar/submicromolar range, but they were all less active as compared to the unsubstituted compounds **5b** and **11b**. The insertion of a chlorine atom (**5d** and **11d**) as an electron-withdrawing group determined a general increase in the inhibitory potency with respect to the insertion of the electron-releasing groups (**5c**, **5e**, **11c**, and **11e**). The presence of bulky *tert*-butyl group (**5e** and **11e**) improved the inhibitory activity when compared to compounds **5c** and **11c**, which bear methyl group.

Compound **11a** represented the most potent molecule among the series. This indicates that the combination of a cyano group and a bulky isobutyl group is favorable for the XO inhibitory activity of 5-phenylisoxazole-3-carboxylic acid derivatives, as previously observed by Ishibuchi et al. for 1-phenylpyrazole inhibitors [6].

2.3. Molecular modeling

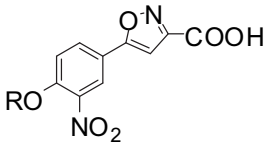
The crystal structures of complexes of xanthine dehydrogenase with febuxostat and Y-700 show a highly specific binding pocket, presenting a long, narrow cavity leading toward the molybdenum–pterin center [4,17]. The molybdenum–pterin sites of both xanthine oxidase and xanthine dehydrogenase are structurally equivalent [18].

To better understand the fairly good XO inhibitory potency of compound **11a** at the molecular level, and to gain an insight into its



Scheme 1. Reagents and conditions: (i) conc. HNO_3, HOAc, 50–60 °C; (ii) isobutyl bromide, K_2CO_3, PEG-400, DMF, 80 °C or alkyl halide, K_2CO_3, KI, DMF, 65 °C; (iii) conc. NH_4OH, I_2, KI, H_2O, r.t.; (iv) alkyl halide, K_2CO_3, KI, DMF, 65 °C; (v) CuCN, DMF, 140 °C; (vi) diethyl oxalate, EtONa, EtOH, −6 °C then r.t.; (vii) $NH_2OH.HCl$, EtOH, reflux; (viii) 4% NaOH solution, THF, 50 °C, then acidified with a 5% HCl solution.

Table 1
In vitro xanthine oxidase inhibitory activity of 5-phenylisoxazole-3-carboxylic acids **5a**–**e**.



Compound	R	IC_{50}[a] (μM)
5a	Isobutyl	1.00
5b	Benzyl	0.97
5c	4-Me-benzyl	n.a.[b]
5d	4-Cl-benzyl	2.83
5e	4-tert-Butyl-benzyl	12.75
Febuxostat		0.003

[a] IC_{50} values: the concentration of the inhibitor required to produce 50% inhibition of xanthine oxidase.
[b] n.a.: not active (less than 50% inhibition at 10 μg/mL).

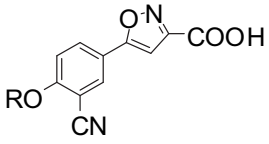
binding mode with XO, experimental docking into the binding pocket of the bovine milk xanthine dehydrogenase/febuxostat complex (PDB entry code 1N5X) [4] was performed. The docking experiment was carried out using the Molegro Virtual Docker [19], and the carboxyl group of compound **11a** was calculated in dissociated form.

It is known from the crystal structures of complexes of xanthine dehydrogenase with febuxostat and Y-700 that they bind xanthine dehydrogenase with the carboxylate moiety interacting with Arg880 and Thr1010, the nitrogen atom of heterocycles forming hydrogen bond with Glu802, and the nitrile group of phenyl unit forming hydrogen bond with Asn768 [4,17]. Accordingly, compound **11a** was expected to bind with XO in a similar way. As shown in Fig. 2, compound **11a** indeed forms hydrogen bonds with Arg880, Thr1010, Glu802, and Asn768. Moreover, additional hydrogen binding between the carboxyl group of Glu802 and the oxygen atom of isoxazole ring occurred.

The isoxazole ring as a whole was sandwiched between Phe914 and Phe1009. The benzonitrile portion was inserted between Leu873 and Leu1014. The hydrophobic 4-isobutoxy tail surrounded by several hydrophobic amino acid residues was positioned at the entrance of the active site-directed channel. All these hydrophobic interactions are very similar to those of febuxostat and Y-700 [4,17] and may contribute to the binding of compound **11a** to XO.

The binding mode of compound **11a** is thus very similar to that of febuxostat and Y-700 [4,17]. This explains the fairly good XO inhibitory potency of compound **11a** at the molecular level.

Table 2
In vitro xanthine oxidase inhibitory activity of 5-phenylisoxazole-3-carboxylic acids **11a**–**e**.



Compound	R	IC_{50}[a] (μM)
11a	Isobutyl	0.36
11b	Benzyl	0.59
11c	4-Me-benzyl	n.a.[b]
11d	4-Cl-benzyl	0.63
11e	4-tert-Butyl-benzyl	1.01
Febuxostat		0.003

[a] IC_{50} values: the concentration of the inhibitor required to produce 50% inhibition of xanthine oxidase.
[b] n.a.: not active (less than 50% inhibition at 10 μg/mL).

However, the potency of compound **11a** to inhibit XO was weaker than that of febuxostat and Y-700 [5,6]. The difference in the positions of nitrogen atom of heterocyclic rings may be one reason. The *N*-3 of febuxostat and *N*-2 of Y-700 are presented at the similar position relative to the phenyl portion, while the nitrogen atom of isoxazole ring of compound **11a** is far from the phenyl portion than that of febuxostat and Y-700, making the nitrogen atom adjacent to the phenyl portion as the preferred orientation. The difference in the steric bulk of the heterocyclic rings may provide another plausible explanation for the potency difference between compound **11a** and febuxostat, as suggested by Fukunari et al. [17]. The C-4 of compound **11a** is smaller than the corresponding sulfur atom of febuxostat, and the compound **11a** also lacks a methyl group, which could introduce additional bulk and hydrophobic interaction. Thus, introducing some groups into the C-4 position to increase its bulky and hydrophobic effects may be favorable for the potency of 5-phenylisoxazole-3-carboxylic acid derivatives against XO.

Lastly, the bovine and human sequences of this enzyme are characterized by 90% identity, and almost all of the amino acids interact with the compound **11a** are conserved in the bovine and human enzymes [20,21]. Therefore, the mechanism of the XO inhibition by the compound **11a** seems applicable to human XO as well.

3. Conclusions

We report the synthesis and in vitro XO inhibitory activity of the 5-phenylisoxazole-3-carboxylic acid derivatives. Among the derivatives synthesized, compounds **5b**, **11a**, **11b**, and **11d** with IC_{50} values lower than 1 μM were obtained. The presence of a cyano group at the 3-position of phenyl moiety turned out to be the preferred substitution pattern, as its transformation into the nitro group determined a general reduction of the inhibitory potency. The present study also points out that the combination of a cyano group and a bulky isobutyl group is preferred for the XO inhibitory activity of 5-phenylisoxazole-3-carboxylic acid derivatives.

Among the xanthine oxidase inhibitors used, allopurinol is the most widely employed. However, adverse side effects associated with the use of allopurinol are sometime very serious and a search for substitutes is therefore highly warranted. The non-purine XO inhibitors related with 5-phenylisoxazole-3-carboxylic acid scaffold might circumvent such problems. Obviously, the toxicological profile of 5-phenylisoxazole-3-carboxylic acid derivatives and their in vivo hypouricemic effects have to be established before their therapeutic potentials can be substantiated. However, these compounds, especially compound **11a**, constitute plausible lead candidates for further investigations.

4. Experimental protocols

4.1. Chemistry

Melting points were recorded via a YRT-3 melting point apparatus, and were uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker ARX-300 spectrometer, and chemical shifts (δ) were given in ppm downfield from TMS and used as the internal standard. Coupling constant (J) values were in Hz. IR spectra were determined as KBr pellets on Bruker IFS-55 spectrometer, and were expressed in cm^{-1}. The progress of the reactions was monitored by TLC using several solvent systems of different polarities. TOF-HRMS spectra were determined on an Agilent 1100 instrument. All solvents and reagents were of analytical grade and used as received.

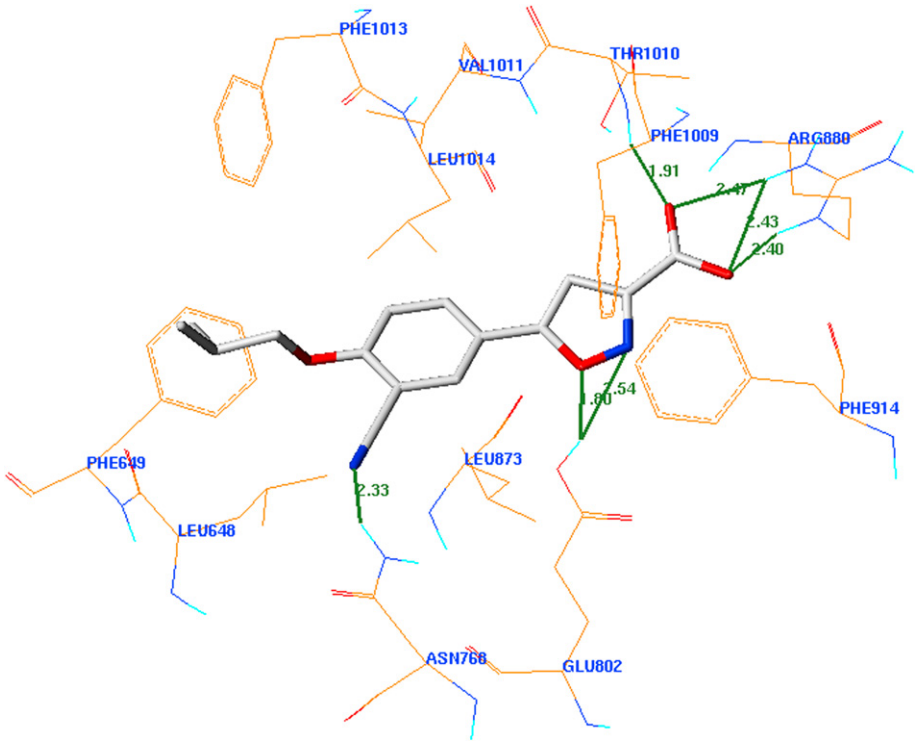


Fig. 2. Amino acid residues interacting with compound **11a** (gray) in the structure of the enzyme inhibitor complex. Hydrogen bonds are represented by the dashed green lines.

*4.1.1. Synthesis of 1-(4-hydroxy-3-nitrophenyl)ethanone (**1**)*

A solution of 1-(4-hydroxyphenyl)ethanone (15 g, 0.11 mol) in acetic acid (40 mL) was heated to 45 °C, to which concentrated nitric acid (10.5 mL, 0.11 mol) was gradually added to maintain the temperature at 50–60 °C. Then, the mixture was stirred at this temperature for 0.5 h, and then poured into ice-cold water (200 mL). The precipitate was filtered, washed with water, and recrystallized from ethanol to give **1** (10.5 g, 52.6%) as a yellow solid, m.p. 131.0–132.6 °C (m.p. 128–130 °C [8]).

*4.1.2. Synthesis of 1-(4-isobutyloxy-3-nitrophenyl)ethanone (**2a**)*

A mixture of compound **1** (15.8 g, 0.087 mol), isobutyl bromide (41.6 g, 0.31 mol), anhydrous K_2CO_3 (42.2 g, 0.31 mol), PEG-400 (4 mL), and DMF (150 mL) was heated under stirring at 80 °C for 7 h. After the reaction was completed, the mixture was poured into water (600 mL), and extracted with methylene dichloride (80 mL × 3). The combined organic layer was washed with a 3% potassium hydroxide solution (100 mL × 2), dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum to obtain a residue. Water (100 mL) was then added to the residue. The precipitate was collected and washed with water to yield **2a** (12.1 g, 58.4%) as a dark yellow solid, m.p. 64.4–66.4 °C. ^{1}H NMR (DMSO-d_6): δ 8.41 (d, 1H, J = 2.2 Hz, Ar–H), 8.20 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.48 (d, 1H, J = 8.9 Hz, Ar–H), 4.04 (d, 2H, J = 6.4 Hz, CH_2), 2.59 (s, 3H, CH_3), 2.12–1.99 (m, 1H, CH), 0.98 (d, 6H, J = 6.7 Hz, $2CH_3$).

*4.1.3. Synthesis of 1-(4-benzyloxy-3-nitrophenyl)ethanone (**2b**)*

A mixture of compound **1** (5 g, 0.028 mol), benzyl chloride (6.4 mL, 0.055 mol), anhydrous K_2CO_3 (11.4 g, 0.083 mol), KI (0.27 g), and DMF (50 mL) was heated under stirring at 65 °C for 3 h. After the reaction was completed, the mixture was poured into water (300 mL). The precipitate was collected by filtration and recrystallized from ethyl acetate to yield **2b** (5 g, 66.8%) as a light yellow solid, m.p. 132.7–134.7 °C (m.p. 134–136 °C [8]).

*4.1.4. Synthesis of 1-[4-(4-methylbenzyloxy)-3-nitrophenyl]ethanone (**2c**)*

Compound **2c** was synthesized in the same manner as that for **2b**, but using 4-methylbenzyl chloride instead of benzyl chloride as a yellow solid with yield of 66%, m.p. 108.8–111.8 °C; ^{1}H NMR ($CDCl_3$): δ 8.40 (d, 1H, J = 2.2 Hz, Ar–H), 8.20 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.56 (d, 1H, J = 8.9 Hz, Ar–H), 7.35 (d, 2H, J = 8.0 Hz, Ar–H), 7.22 (d, 2H, J = 7.9 Hz, Ar–H), 5.37 (s, 2H, CH_2), 2.58 (s, 3H, CH_3), 2.31(s, 3H, CH_3).

*4.1.5. Synthesis of 1-[4-(4-chlorobenzyloxy)-3-nitrophenyl]ethanone (**2d**)*

Compound **2d** was synthesized in the same manner as that for **2b**, but using 4-chlorobenzyl chloride instead of benzyl chloride as a light yellow solid with yield of 53.3%, m.p. 118.7–120.0 °C. ^{1}H NMR (DMSO-d_6): δ 8.42 (d, 1H, J = 2.1 Hz, Ar–H), 8.22 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.56 (d, 1H, J = 8.9 Hz, Ar–H), 7.50 (s, 4H, Ar–H), 5.43 (s, 2H, CH_2), 2.59 (s, 3H, CH_3).

*4.1.6. Synthesis of 1-[4-(4-tert-butylbenzyloxy)-3-nitrophenyl]ethanone (**2e**)*

Compound **2e** was synthesized in the same manner as that for **2b**, but using 4-*tert*-butylbenzyl chloride instead of benzyl chloride as a light yellow solid with yield of 59.8%, m.p. 96.1–97.3 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 8.40 (d, 1H, J = 2.1 Hz, Ar–H), 8.21 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.58 (d, 1H, J = 8.9 Hz, Ar–H), 7.42 (m, 4H, Ar–H), 5.38 (s, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.28 (s, 9H, $3CH_3$).

*4.1.7. General procedure for the synthesis of compounds **3a–e***

Sodium metal (0.31 g, 13.4 mmol) was cautiously added to absolute ethanol (15 mL) gave a solution of sodium ethoxide, to which a solution of diethyl oxalate (1.78 g, 12.2 mmol) and the corresponding **2** (12.2 mmol) in THF (25 mL) was added dropwise under stirring at −6 °C. The mixture was stirred at the same temperature for 1 h, and was then stirred at room temperature for

4 h. After the reaction was completed, the solvent was removed under reduced pressure. Water (80 mL) was added to the residue, which was acidified with a 5% HCl solution to pH 3. The resulting precipitate was collected by filtration and recrystallized to yield the desired product.

*4.1.7.1. (Z)-Ethyl 2-hydroxy-4-(4-isobutoxy-3-nitrophenyl)-4-oxobut-2-enoate (**3a**).* Yield of 55.8%, a white solid, m.p. 102.5–104.1 °C (from ethanol); ^{1}H NMR ($CDCl_3$): δ 15.14 (br s, 1H, OH), 8.48 (d, 1H, J = 2.2 Hz, Ar–H), 8.17 (dd, 1H, J = 2.3 and 8.9 Hz, Ar–H), 7.16 (d, 1H, J = 8.9 Hz, Ar–H), 7.02 (s, 1H, olefin-H), 4.42 (q, 2H, J = 7.1 Hz, CH_2CH_3), 3.96 (d, 2H, J = 6.4 Hz, CH_2), 2.24–2.15 (m, 1H, CH), 1.42 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.08 (d, 6H, J = 6.7 Hz, $2CH_3$).

*4.1.7.2. (Z)-Ethyl 4-(4-benzyloxy-3-nitrophenyl)-2-hydroxy-4-oxobut-2-enoate (**3b**).* Yield of 36.5%, a light yellow solid, m.p. 116.4–117.4 °C (from ethanol); ^{1}H NMR ($CDCl_3$): δ 15.13 (br s, 1H, OH), 8.49 (d, 1H, J = 2.3 Hz, Ar–H), 8.15 (dd, 1H, J = 2.3 and 8.9 Hz, Ar–H), 7.47–7.36 (m, 5H, Ar–H), 7.23 (d, 1H, J = 8.9 Hz, Ar–H), 7.01 (s, 1H, olefin-H), 5.35 (s, 2H, CH_2), 4.41 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.42 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.7.3. (Z)-Ethyl 2-hydroxy-4-[4-(4-methylbenzyloxy)-3-nitrophenyl]-4-oxobut-2-enoate (**3c**).* Yield of 41.5%, a light yellow solid, m.p. 109.4–111.2 °C (from ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 8.24 (d, 1H, J = 1.6 Hz, Ar–H), 8.03 (d, 1H, J = 8.6 Hz, Ar–H), 7.44 (d, 1H, J = 8.6 Hz, Ar–H), 7.35 (d, 2H, J = 7.7 Hz, Ar–H), 7.21 (d, 2H, J = 7.8 Hz, Ar–H), 6.25 (s, 1H, olefin-H), 5.29 (s, 2H, CH_2), 4.10 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.31 (s, 3H, CH_3), 1.23 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.7.4. (Z)-Ethyl 4-[4-(4-chlorobenzyloxy)-3-nitrophenyl]-2-hydroxy-4-oxobut-2-enoate (**3d**).* Yield of 49.0%, a yellow solid, m.p. 130.9–132.1 °C (from acetone-ethanol); ^{1}H NMR (DMSO-d_6): δ 8.61 (s, 1H, Ar–H), 8.38 (d, 1H, J = 8.4 Hz, Ar–H), 7.60 (d, 1H, J = 8.4 Hz, Ar–H), 7.50 (s, 4H, Ar–H), 7.18 (s, 1H, olefin-H), 5.45 (s, 2H, CH_2), 4.32 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.32 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.7.5. (Z)-Ethyl 4-[4-(4-tert-butylbenzyloxy)-3-nitrophenyl]-2-hydroxy-4-oxobut-2-enoate (**3e**).* Yield of 38.3%, a yellow solid, m.p. 128.3–130.0 °C (from ethyl acetate); ^{1}H NMR (DMSO-d_6): δ 8.49 (d, 1H, J = 2.1 Hz, Ar–H), 8.23 (dd, 1H, J = 2.1 and 8.8 Hz, Ar–H), 7.63 (d, 1H, J = 9.0 Hz, Ar–H), 7.50 (s, 5H, Ar–H, olefin–H), 5.31 (s, 2H, CH_2), 4.39 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.25 (m, 12H, $3CH_3$, CH_2CH_3).

*4.1.8. General procedure for the synthesis of compounds **4a–e***

A mixture of the corresponding **3** (5.93 mmol), hydroxylamine hydrochloride (0.46 g, 5.93 mmol), and anhydrous ethanol (60 mL) was heated under reflux for 6 h. After the reaction was completed, about one-third of the solvent was evaporated, and the residue was let to stand at room temperature for 2 h to complete the precipitation. The precipitate was collected by filtration, washed with ethanol, and recrystallized to yield the desired product.

*4.1.8.1. Ethyl 5-(4-isobutoxy-3-nitrophenyl)isoxazole-3-carboxylate (**4a**).* Yield of 80.7%, a white solid, m.p. 134.8–136.1 °C (from ethanol); ^{1}H NMR ($CDCl_3$): δ 8.27 (d, 1H, J = 2.2 Hz, Ar–H), 7.95 (dd, 1H, J = 2.3 and 8.8 Hz, Ar–H), 7.18 (d, 1H, J = 8.9 Hz, Ar–H), 6.91 (s, 1H, 4-H), 4.48 (q, 2H, J = 7.1 Hz, CH_2CH_3), 3.94 (d, 2H, J = 6.4 Hz, CH_2), 2.24–2.15 (m, 1H, CH), 1.45 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.08 (d, 6H, J = 6.7 Hz, $2CH_3$).

*4.1.8.2. Ethyl 5-(4-benzyloxy-3-nitrophenyl)isoxazole-3-carboxylate (**4b**).* Yield of 86.4%, a white solid, m.p. 171.8–173.0 °C (from ethanol); ^{1}H NMR (DMSO-d_6): δ 8.51 (s, 1H, Ar–H), 8.23 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.65 (d, 1H, J = 8.9 Hz, Ar–H), 7.58 (s, 1H, 4-H), 7.49–7.36 (m, 5H, Ar–H), 5.42 (s, 2H, CH_2), 4.40 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.34 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.8.3. Ethyl 5-[4-(4-methylbenzyloxy)-3-nitrophenyl]isoxazole-3-carboxylate (**4c**).* Yield of 72.0%, a white solid, m.p. 189.0–189.7 °C (from acetonitrile); ^{1}H NMR (DMSO-d_6): δ 8.49 (d, 1H, J = 2.2 Hz, Ar–H), 8.21 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.63 (d, 1H, J = 9.0 Hz, Ar–H), 7.56 (s, 1H, 4-H), 7.35 (d, 2H, J = 8.0 Hz, Ar–H), 7.22 (d, 2H, J = 7.9 Hz, Ar–H), 5.36 (s, 2H, CH_2), 4.40 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.31 (s, 3H, CH_3), 1.34 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.8.4. Ethyl 5-[4-(4-chlorobenzyloxy)-3-nitrophenyl]isoxazole-3-carboxylate (**4d**).* Yield of 86.4%, a white solid, m.p. 174.3–175.2 °C (from ethanol); ^{1}H NMR (DMSO-d_6): δ 8.51 (d, 1H, J = 2.1 Hz, Ar–H), 8.24 (dd, 1H, J = 2.1 and 8.8 Hz, Ar–H), 7.63 (d, 1H, J = 9.0 Hz, Ar–H), 7.58 (s, 1H, 4-H), 7.50 (s, 4H, Ar–H), 5.42 (s, 2H, CH_2), 4.40 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.34 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.8.5. Ethyl 5-[4-(4-tert-butylbenzyloxy)-3-nitrophenyl)]isoxazole-3-carboxylate (**4e**).* Yield of 71.4%, a white solid, m.p. 165.5–166.4 °C (from ethanol); ^{1}H NMR (DMSO-d_6): δ 8.50 (d, 1H, J = 2.2 Hz, Ar–H), 8.23 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.65 (d, 1H, J = 9.0 Hz, Ar–H), 7.58 (s, 1H, 4-H), 7.42 (q, 4H, J = 8.4 Hz, Ar–H), 5.37 (s, 2H, CH_2), 4.40 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.34 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.28 (s, 9H, $3CH_3$).

*4.1.9. General procedure for the synthesis of compounds **5a–e***

A mixture of the corresponding **4** (2.33 mmol), 4% NaOH solution (23.3 mL), and THF (10 mL) was heated at 50 °C for 1.5 h. After the reaction was completed, the solvent was then removed under reduced pressure. The resulting pasty residue was dissolved in water (80 mL) and acidified with a 5% HCl solution to pH 1. The suspension was stirred at room temperature for 20 min. The solid was collected by filtration, washed with water, and recrystallized to yield the desired product.

*4.1.9.1. 5-(4-isobutoxy-3-nitrophenyl)isoxazole-3-carboxylic acid (**5a**).* Yield of 41.0%, a light yellow solid, m.p. 181.3–182.2 °C (from methanol); ^{1}H NMR (DMSO-d_6): δ 14.1 (br s, 1H, COOH), 8.46 (d, 1H, J = 2.1 Hz, Ar–H), 8.19 (dd, 1H, J = 2.1 and 8.8 Hz, Ar–H), 7.54 (d, 1H, J = 8.9 Hz, Ar–H), 7.48 (s, 1H, 4-H), 4.04 (d, 2H, J = 6.4 Hz, CH_2), 2.12–1.99 (m, 1H, CH), 0.99 (d, 6H, J = 6.7 Hz, $2CH_3$); IR (KBr, cm^{-1}): 3453, 2964, 1720, 1624, 1531, 1447,1397, 1384, 1286, 1167, 1001; TOF-HRMS m/z = 329.0739 $[M + Na]^+$($C_{14}H_{14}N_2NaO_6$ requires 329.0750).

*4.1.9.2. 5-(4-benzyloxy-3-nitrophenyl)isoxazole-3-carboxylic acid (**5b**).* Yield of 67%, a light yellow solid, m.p. 189.6–191.5 °C (from acetic acid); ^{1}H NMR (DMSO-d_6): δ 8.48 (d, 1H, J = 2.0 Hz, Ar–H), 8.21 (dd, 1H, J = 2.0 and 8.8 Hz, Ar–H), 7.64 (d, 1H, J = 8.9 Hz, Ar–H), 7.49–7.36 (m, 6H, 4-H, Ar–H), 5.42 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3453, 2926, 1711, 1625, 1531, 1496, 1445, 1385, 1356, 1301, 1268; TOF-HRMS m/z = 363.0593 $[M + Na]^+$($C_{17}H_{12}N_2NaO_6$ requires 363.0593).

*4.1.9.3. 5-[4-(4-methylbenzyloxy)-3-nitrophenyl]isoxazole-3-carboxylic acid (**5c**).* Yield of 61%, a light yellow solid, m.p. 173.2–174.7 °C (from methanol); ^{1}H NMR (DMSO-d_6): δ 8.47 (d, 1H, J = 2.1 Hz, Ar–H), 8.20 (dd, 1H, J = 2.4 and 9.3 Hz, Ar–H), 7.63 (d, 1H, J = 9.0 Hz, Ar–H), 7.49 (s, 1H, 4-H), 7.35 (d, 2H, J = 8.0 Hz, Ar–H), 7.22 (d, 2H, J = 8.1 Hz, Ar–H), 5.36 (s, 2H, CH_2), 2.31 (s, 3H, CH_3); IR (KBr, cm^{-1}): 3511, 3139, 2920, 1725, 1624, 1531, 1449, 1384, 1277, 1173, 996; TOF-HRMS m/z = 377.0738 $[M + Na]^+$($C_{18}H_{14}N_2NaO_6$ requires 377.0750).

*4.1.9.4. 5-[4-(4-chlorobenzyloxy)-3-nitrophenyl]isoxazole-3-carboxylic acid (**5d**).* Yield of 25.8%, a light yellow solid, m.p. 195.5–196.9 °C (from ethanol–THF); ^{1}H NMR (DMSO-d_6): δ 8.49 (d, 1H, J = 2.1 Hz, Ar–H), 8.23 (dd, 1H, J = 2.1 and 8.8 Hz, Ar–H), 7.62 (d, 1H, J = 9.0 Hz, Ar–H), 7.50 (s, 5H, 4-H, Ar–H), 5.42 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3487, 3145,1719, 1624, 1533, 1494, 1444, 1304, 1264, 1011; TOF-HRMS m/z = 397.0196 $[M + Na]^+$($C_{17}H_{11}ClN_2NaO_6$ requires 397.0204).

*4.1.9.5. 5-[4-(4-tert-butylbenzyloxy)-3-nitrophenyl]isoxazole-3-carboxylic acid (**5e**).* Yield of 62.5%, a yellow solid, m.p. 158.5–159.8 °C (from ethanol); ^{1}H NMR (DMSO-d_6): δ 8.47 (d, 1H, J = 2.2 Hz, Ar–H), 8.21 (dd, 1H, J = 2.2 and 8.8 Hz, Ar–H), 7.65 (d, 1H, J = 9.0 Hz, Ar–H), 7.49–7.38 (m, 5H, 4-H, Ar–H), 5.37 (s, 2H, CH_2), 1.28 (s, 9H, $3CH_3$); IR (KBr, cm^{-1}): 3458, 3143, 2962, 1732, 1625, 1596, 1532, 1449, 1384, 1284; TOF-HRMS m/z = 419.1218 $[M + Na]^+$($C_{21}H_{20}N_2NaO_4$ requires 419.1219).

*4.1.10. Synthesis of 1-(4-hydroxy-3-iodophenyl)ethanone (**6**)*

To a solution of KI (60.1 g, 0.37 mol) and I_2 (18.7 g, 0.074 mol) in water (160 mL), 1-(4-hydroxyphenyl)ethanone (10 g, 0.074 mol) in concentrated NH_4OH (500 mL) was added rapidly with stirring. The resulting mixture was stirred overnight at room temperature (color changed from gray to yellow). After the reaction was completed, the mixture was filtered. The filtrate was acidified with concentrated HCl solution, and the yellow precipitate was collected by filtration. The crude product was recrystallized from methanol–water (7:3) to afford **6** (10.8 g, 56.1%) as a yellow solid, m.p. 153.4–156.0 °C (m.p. 153–155 °C [9]).

*4.1.11. Synthesis of 1-(4-isobutoxy-3-iodophenyl)ethanone (**7a**)*

Similar to the procedure used to prepare **2b**, reaction of **6** with isobutyl bromide provided **7a** in a yield of 70.2% as a light yellow solid, m.p. 50.6–51.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.38 (d, 1H, J = 2.2 Hz, Ar–H), 7.91 (dd, 1H, J = 2.2 and 8.6 Hz, Ar–H), 6.79 (d, 1H, J = 8.6 Hz, Ar–H), 3.84 (d, 2H, J = 6.3 Hz, CH_2), 2.54 (s, 3H, CH_3), 2.24–2.11 (m, 1H, CH), 1.10 (d, 6H, J = 6.7 Hz, $2CH_3$).

*4.1.12. Synthesis of 1-(4-benzyloxy-3-iodophenyl)ethanone (**7b**)*

Similar to the procedure used to prepare **2b**, reaction of **6** with benzyl chloride provided **7b** in a yield of 44.7% as a yellow solid, m.p. 126.0–127.3 °C (from ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.33 (d, 1H, J = 2.1 Hz, Ar–H), 7.98 (dd, 1H, J = 2.1 and 8.6 Hz, Ar–H), 7.52–7.32 (m, 5H, Ar–H), 7.19 (d, 1H, J = 8.7 Hz, Ar–H), 5.32 (s, 2H, CH_2), 2.53 (s, 3H, CH_3).

*4.1.13. Synthesis of 1-[3-iodo-4-(4-methylbenzyloxy)-phenyl]ethanone (**7c**)*

Similar to the procedure used to prepare **2b**, reaction of **6** with 4-methylbenzyl chloride provided **7c** in a yield of 53.7% as a light yellow solid, m.p. 117.3–118.9 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 8.40 (d, 1H, J = 2.0 Hz, Ar–H), 7.90 (dd, 1H, J = 2.0 and 8.6 Hz, Ar–H), 7.36 (d, 2H, J = 7.9 Hz, Ar–H), 7.20 (d, 2H, J = 7.8 Hz, Ar–H), 6.86 (d, 1H, J = 8.6 Hz, Ar–H), 5.18 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.36 (s, 3H, CH_3).

*4.1.14. Synthesis of 1-[4-(4-chlorobenzyloxy)-3-iodophenyl]ethanone (**7d**)*

Similar to the procedure used to prepare **2b**, reaction of **6** with 4-chlorobenzyl chloride provided **7d** in a yield of 73.4% as a light yellow solid, m.p. 126.5–127.7 °C (from ethanol–chloroform). ^{1}H NMR ($CDCl_3$): δ 8.40 (d, 1H, J = 2.1 Hz, Ar–H), 7.92 (dd, 1H, J = 2.1 and 8.6 Hz, Ar–H), 7.43 (d, 2H, J = 8.6 Hz, Ar–H),7.38 (d, 2H, J = 8.6 Hz, Ar–H), 6.85 (d, 1H, J = 8.6 Hz, Ar–H), 5.18 (s, 2H, CH_2), 2.54 (s, 3H, CH_3).

*4.1.15. Synthesis of 1-[4-(4-tert-butylbenzyloxy)-3-iodophenyl]ethanone (**7e**)*

Similar to the procedure used to prepare **2b**, reaction of **6** with 4-*tert*-butylbenzyl chloride provided **7e** in a yield of 66.9% as a yellow solid, m.p. 107.9–108.8 °C (from ethanol). ^{1}H NMR ($CDCl_3$): δ 8.41 (d, 1H, J = 2.1 Hz, Ar–H), 7.91 (dd, 1H, J = 2.1 and 8.6 Hz, Ar–H), 7.42 (s, 4H, Ar–H), 6.89 (d, 1H, J = 8.6 Hz, Ar–H), 5.20 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 1.33 (s, 9H, $3CH_3$).

*4.1.16. General procedure for the synthesis of compounds **8a–e***

A mixture of the corresponding **7** (18.3 mmol), cuprous cyanide (2.63 g, 29.3 mmol), and DMF (50 mL) was heated at 140 °C for 7 h. After the reaction was completed, the mixture was cooled to room temperature, poured into water (200 mL), and stirred at room temperature for 0.5 h. The formed precipitate was collected by filtration and dried at room temperature to give the crude product as a solid. The solid was extracted with hot chloroform (100 mL) and filtrated. The filtrate was evaporated under reduced pressure, and the residue was then recrystallized to yield the desired product.

*4.1.16.1. 1-(3-cyano-4-isobutoxyphenyl)ethanone (**8a**).* Yield of 58.4%, a white solid, m.p. 97.1–97.9 °C (from ethanol); ^{1}H NMR (DMSO-d_6): δ 8.34 (d, 1H, J = 2.2 Hz, Ar–H), 8.20 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.37 (d, 1H, J = 8.9 Hz, Ar–H), 4.03 (d, 2H, J = 6.5 Hz, CH_2), 2.57 (s, 3H, CH_3), 2.16–2.03 (m, 1H, CH), 1.02 (d, 6H, J = 6.7 Hz, 2 CH_3).

*4.1.16.2. 1-(4-benzyloxy-3-cyanophenyl)ethanone (**8b**).* Yield of 66.7%, a light yellow solid, m.p. 126.5–127.3 °C (from ethanol); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.33 (s, 1H, Ar–H), 7.98 (d, 1H, Ar–H), 7.52–7.32 (m, 6H, Ar–H), 5.40 (s, 2H, CH_2), 2.57 (s, 3H, CH_3).

*4.1.16.3. 1-[3-cyano-4-(4-methylbenzyloxy)phenyl]ethanone (**8c**).* Yield of 52.2%, a dark yellow solid, m.p. 102.9–103.8 °C (from ethanol); ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 1H, J = 2.2 Hz, Ar–H), 8.10 (dd, 1H, J = 2.3 and 8.9 Hz, Ar–H), 7.33 (d, 2H, J = 8.0 Hz, Ar–H), 7.20 (d, 2H, J = 8.0 Hz, Ar–H), 7.06 (d, 1H, J = 8.9 Hz, Ar–H), 5.26 (s, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.36 (s, 3H, CH_3).

*4.1.16.4. 1-[4-(4-chlorobenzyloxy)-3-cyanophenyl]ethanone (**8d**).* Yield of 79.8%, a light yellow solid, m.p. 119.6–120.9 °C; ^{1}H NMR ($CDCl_3$): δ 8.20 (d, 1H, J = 2.2 Hz, Ar–H), 8.12 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.39 (s, 4H, Ar–H), 7.05 (d, 1H, J = 8.9 Hz, Ar–H), 5.26 (s, 2H, CH_2), 2.57 (s, 3H, CH_3).

*4.1.16.5. 1-[4-(4-tert-butylbenzyloxy)-3-cyanophenyl]ethanone (**8e**).* Purification by flash silica gel column chromatography using ethyl acetate as eluting solvent provided **8e** in a yield of 70.9% as a yellow solid, m.p. 106.9–108.3 °C. ^{1}H NMR ($CDCl_3$): δ 8.19 (d, 1H, J = 2.2 Hz, Ar–H), 8.11(dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.43 (d, 2H, J = 8.5 Hz, Ar–H), 7.38 (d, 2H, J = 8.4 Hz, Ar–H), 7.08 (d, 1H, J = 8.9 Hz, Ar–H), 5.26 (s, 2H, CH_2), 2.56 (s, 3H, CH_3), 1.33 (s, 9H, $3CH_3$).

*4.1.17. (Z)-Ethyl 4-(3-cyano-4-isobutoxyphenyl)-2-hydroxy-4-oxobut-2-enoate (**9a**)*

Similar to the procedure used to prepare **3**, reaction of diethyl oxalate with **8a** provided **9a** in a yield of 55.2% as a white solid, m.p. 106.7–107.3 °C (from ethanol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 15.06 (br s, 1H, OH), 8.23 (d, 1H, J = 2.2 Hz, Ar–H), 8.18 (dd, 1H, J = 2.3 and 8.9 Hz, Ar–H), 7.06 (d, 1H, J = 8.9 Hz, Ar–H), 6.99 (s, 1H, olefin-H), 4.41 (q, 2H, J = 7.1 Hz, CH_2CH_3), 3.94 (d, 2H, J = 6.5 Hz, CH_2), 2.29–2.16 (m, 1H, CH), 1.42 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.10 (d, 6H, J = 6.8 Hz, $2CH_3$).

*4.1.18. (Z)-Ethyl 4-(4-benzyloxy-3-cyanophenyl)-2-hydroxy-4-oxobut-2-enoate (**9b**)*

Similar to the procedure used to prepare **3**, reaction of diethyl oxalate with **8b** provided **9b** in a yield of 52.3% as a white solid, m.p. 125.2–126.3 °C (from ethanol–ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.56 (s, 1H, Ar–H), 8.36 (d, 1H, J = 8.2 Hz, Ar–H), 7.52–7.35 (m, 6H, Ar–H), 7.17 (s, 1H, olefin-H), 5.42 (s, 2H, CH_2), 4.31 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.31 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.19. (Z)-Ethyl 4-[3-cyano-4-(4-methylbenzyloxy)phenyl]-2-hydroxy-4-oxobut-2-enoate (**9c**)*

Similar to the procedure used to prepare **3**, reaction of diethyl oxalate with **8c** provided **9c** in a yield of 66.6% as a dark yellow solid, m.p. 154.2–155.4 °C (from ethanol–ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 8.56 (s, 1H, Ar–H), 8.35 (d, 1H, J = 8.5 Hz, Ar–H), 7.50 (d, 1H, J = 9.0 Hz, Ar–H), 7.39 (d, 2H, J = 7.7 Hz, Ar–H), 7.24 (d, 2H, J = 7.7 Hz, Ar–H), 7.19 (s, 1H, olefin-H), 5.36 (s, 2H, CH_2), 4.32 (q, 2H, J = 7.0 Hz, CH_2CH_3), 2.32 (s, 3H, CH_3), 1.32 (t, 3H, J = 7.0 Hz, CH_2CH_3).

*4.1.20. (Z)-Ethyl 4-[4-(4-chlorobenzyloxy)-3-cyanophenyl]-2-hydroxy-4-oxobut-2-enoate (**9d**)*

Similar to the procedure used to prepare **3**, reaction of diethyl oxalate with **8d** provided **9d** in a yield of 59.7% as a yellow brown solid, m.p. 132.2–133.1 °C (ethanol–ethyl acetate). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (s, 1H, Ar–H), 8.37 (d, 1H, J = 8.9 Hz, Ar–H), 7.52 (s, 4H, Ar–H), 7.48 (s, 1H, Ar–H), 7.20 (s, 1H, olefin–H), 5.42 (s, 2H, CH_2), 4.32 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.32 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.21. (Z)-Ethyl 4-[4-(4-tert-butylbenzyloxy)-3-cyanophenyl]-2-hydroxy-4-oxobut-2-enoate (**9e**)*

Similar to the procedure used to prepare **3**, reaction of diethyl oxalate with **8e** provided **9e** in a yield of 39.4% as a yellow brown solid, m.p. 110.9–112.1 °C (from acetic acid). ^{1}H NMR (DMSO-d_6): δ 8.57 (s, 1H, Ar–H), 8.37 (d, 1H, J = 8.7 Hz, Ar–H), 7.52 (d, 1H, J = 9.1 Hz, Ar–H), 7.46 (d, 2H, J = 8.5 Hz, Ar–H), 7.42 (d, 2H, J = 8.5 Hz, Ar–H), 7.20 (s, 1H, olefin-H), 5.38 (s, 2H, CH_2), 4.32 (q, 2H, J = 7.0 Hz, CH_2CH_3), 1.34–1.29 (m, 12H, CH_2CH_3, $3CH_3$).

*4.1.22. Ethyl 5-(3-cyano-4-isobutoxyphenyl)isoxazole-3-carboxylate (**10a**)*

Similar to the procedure used to prepare **4**, reaction of hydroxylamine hydrochloride with **9a** provided **10a** in a yield of 85.4% as a white solid, m.p. 176.6–177.5 °C. ^{1}H NMR (300 MHz, DMSO): δ 8.39 (d, 1H, J = 2.3 Hz, Ar–H), 8.21 (dd, 1H, J = 2.3 and 8.9 Hz, Ar–H), 7.51(s, 1H, H-4), 7.42 (d, 1H, J = 9.0 Hz, Ar–H), 4.39 (q, 2H, J = 7.1 Hz, CH_2CH_3), 4.02 (d, 2H, J = 6.5 Hz, CH_2), 2.16–2.03 (m, 1H, CH), 1.34 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.02 (d, 6H, J = 7.3 Hz, $2CH_3$).

*4.1.23. Ethyl 5-[4-(benzyloxy)-3-cyanophenyl]isoxazole-3-carboxylate (**10b**)*

Similar to the procedure used to prepare **4**, reaction of hydroxylamine hydrochloride with **9b** provided **10b** in a yield of 86.5% as a light yellow solid, m.p. 184.0–184.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 (d, 1H, J = 2.2 Hz, Ar–H), 7.92 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.47–7.35 (m, 5H, Ar–H), 7.13 (d, 1H, J = 8.9 Hz, Ar–H), 6.87 (s, 1H, H-4), 5.30 (s, 2H, CH_2), 4.47 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.44 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.24. Ethyl 5-[3-cyano-4-(4-methylbenzyloxy)phenyl]isoxazole-3-carboxylate (**10c**)*

Similar to the procedure used to prepare **4**, reaction of hydroxylamine hydrochloride with **9c** provided **10c** in a yield of 63.7% as a white solid, m.p. 190.3–191.5 °C (from ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, 1H, J = 2.2 Hz, Ar–H), 7.91 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.34 (d, 2H, J = 8.0 Hz, Ar–H), 7.21 (d, 2H, J = 8.0 Hz, Ar–H), 7.15 (d, 1H, J = 8.9 Hz, Ar–H), 6.86 (s, 1H, H-4), 5.26 (s, 2H, CH_2), 4.47 (q, 2H, J = 7.1 Hz, CH_2CH_3), 2.36 (s, 3H, CH_3), 1.45 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.25. Ethyl 5-[4-(4-chlorobenzyloxy)-3-cyanophenyl]isoxazole-3-carboxylate (**10d**)*

Similar to the procedure used to prepare **4**, reaction of hydroxylamine hydrochloride with **9d** provided **10d** in a yield of 76.7% as a white solid, m.p. 175.1–175.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, 1H, J = 2.2 Hz, Ar–H), 7.95 (dd, 1H, J = 2.2 and 8.9 Hz, Ar–H), 7.40 (s, 4H, Ar–H), 7.11 (d, 1H, J = 8.9 Hz, Ar–H), 6.88 (s, 1H, H-4), 5.26 (s, 2H, CH_2), 4.47 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.45 (t, 3H, J = 7.1 Hz, CH_2CH_3).

*4.1.26. Ethyl 5-[4-(4-tert-butylbenzyloxy)-3-cyanophenyl]-isoxazole-3-carboxylate (**10e**)*

Similar to the procedure used to prepare **4**, reaction of hydroxylamine hydrochloride with **9e** provided **10e** in a yield of 83.2% as a yellow solid, m.p. 181.3–182.2 °C. ^{1}H NMR (DMSO-d_6): δ 8.42 (s, 1H, Ar–H), 8.24 (d, 1H, J = 8.9 Hz, Ar–H), 7.54 (d, 2H, Ar–H, H-4), 7.48–7.41 (m, 4H, Ar–H), 5.35 (s, 2H, CH_2), 4.40 (q, 2H, J = 7.1 Hz, CH_2CH_3), 1.37–1.29 (m, 12H, CH_2CH_3, $3CH_3$).

*4.1.27. 5-(3-Cyano-4-isobutoxyphenyl)isoxazole-3-carboxylic acid (**11a**)*

Similar to the procedure used to prepare **5**, hydrolysis of **10a** provided **11a** in a yield of 88.7% as a white solid, m.p. 180.0–180.5 °C (from methanol). ^{1}H NMR (DMSO-d_6): δ 14.07 (br s, 1H, COOH), 8.37 (d, 1H, J = 2.1 Hz, Ar–H), 8.20 (dd, 1H, J = 2.1 and 8.9 Hz, Ar–H), 7.44–7.41 (m, 2H, Ar–H, H-4), 4.02 (d, 2H, J = 6.5 Hz, CH_2), 2.16–2.03 (m, 1H, CH), 1.02 (d, 6H, J = 6.7 Hz, $2CH_3$); IR (KBr, cm^{-1}): 3543, 2228, 1730, 1618, 1495, 1451, 1385, 1302, 1264, 1177, 1123; TOF-HRMS m/z = 287.1018 $[M+H]^+$($C_{15}H_{15}N_2O_4$ requires 287.1033).

*4.1.28. 5-(4-Benzyloxy-3-cyanophenyl)isoxazole-3-carboxylic acid (**11b**)*

Similar to the procedure used to prepare **5**, hydrolysis of **10b** provided **11b** in a yield of 65.5% as a white solid, m.p. 183.4–184.1 °C (from acetic acid). ^{1}H NMR (DMSO-d_6): δ 8.40 (d, 1H, J = 2.1 Hz, Ar–H), 8.22 (dd, 1H, J = 2.1 and 8.9 Hz, Ar–H), 7.53(d, 1H, J = 8.9 Hz, Ar–H), 7.50–7.38 (m, 6H, Ar–H, H-4), 5.39 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3423, 2225, 1708, 1616, 1490, 1455, 1385, 1295, 1261, 1179, 1121; TOF-HRMS m/z = 343.0684 $[M+Na]^+$($C_{18}H_{12}N_2NaO_4$ requires 343.0695).

*4.1.29. 5-[3-Cyano-4-(4-methylbenzyloxy)phenyl]-isoxazole-3-carboxylic acid (**11c**)*

Similar to the procedure used to prepare **5**, hydrolysis of **10c** provided **11c** in a yield of 63.4% as a white solid, m.p. 194.7–195.1 °C (from ethanol–THF). ^{1}H NMR (DMSO-d_6): δ 8.37 (s, 1H, Ar–H), 8.20 (d, 1H, J = 8.8 Hz, Ar–H), 7.51 (d, 1H, J = 8.9 Hz, Ar–H), 7.42 (s, 1H, H-4), 7.39 (d, 2H, J = 7.9 Hz, Ar–H), 7.23 (d, 2H, J = 7.8 Hz, Ar–H), 5.33 (s, 2H, CH_2), 2.31 (s, 3H, CH_3); IR (KBr, cm^{-1}): 3423, 2225, 1729, 1616, 1490, 1453, 1385, 1295, 1270, 1179, 1121; TOF-HRMS m/z = 357.0838 $[M+Na]^+$($C_{19}H_{14}N_2NaO_4$ requires 357.0852).

*4.1.30. 5-[4-(4-Chlorobenzyloxy)-3-cyanophenyl]isoxazole-3-carboxylic acid (**11d**)*

Similar to the procedure used to prepare **5**, hydrolysis of **10d** provided **11d** in a yield of 68.1% as a light yellow solid, m.p. 191.5–192.3 °C (from ethanol–THF). ^{1}H NMR (DMSO-d_6): δ 8.40 (d, 1H, J = 2.1 Hz, Ar–H), 8.22 (dd, 1H, J = 2.1 and 8.9 Hz, Ar–H), 7.55–7.50 (m, 5H, Ar–H), 7.43 (s, 1H, H-4), 5.39 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3474, 2223, 1731, 1617, 1496, 1454, 1385, 1291, 1269, 1242, 1124; TOF-HRMS m/z = 377.0300 $[M+Na]^+$($C_{18}H_{11}ClN_2NaO_4$ requires 377.0305).

4.1.31. 5-[4-(4-Tert-butylbenzyloxy)-3-cyanophenyl]isoxazole-3-carboxylic acid (**11e**)

Similar to the procedure used to prepare **5**, hydrolysis of **10e** provided **11e** in a yield of 73.5% as a yellow solid, m.p. 165.0–166.1 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 8.39 (d, 1H, $J = 2.2$ Hz, Ar–H), 8.22 (dd, 1H, $J = 2.3$ and 8.9 Hz, Ar–H), 7.54 (d, 1H, $J = 9.1$ Hz, Ar–H), 7.48–7.40 (m, 5H, Ar–H, H-4), 5.34 (s, 2H, CH_2), 1.29 (s, 9H, $3CH_3$); IR (KBr, cm^{-1}): 3453, 2229, 1732, 1692, 1618, 1501, 1451, 1384, 1302, 1277, 1123; TOF-HRMS $m/z = 399.1313$ $[M + Na]^+$($C_{22}H_{20}N_2NaO_4$ requires 399.1321).

4.2. Assay of the in vitro xanthine oxidase inhibitory activity

The xanthine oxidase activity with xanthine as the substrate was measured spectrophotometrically, based on the procedure reported by Kalra et al. [15] and Tamta et al. [16], with modification. The 200 μL assay mixture consisted of 50 mM phosphate buffer (pH 7.5), 20 μM xanthine (Sigma, X7375), optimal xanthine oxidase (Sigma, X4875), and the test compound. After incubation at 25 °C for 30 min, the reaction was monitored at 295 nm on a SpectraMax Plus 384 reader (MD, USA). The test compounds were initially assayed for their inhibition of xanthine oxidase at a concentration of 10 μg/mL. If an inhibition of more than 50% was observed, the compound was classified as active. The active compounds were consequently tested at eight concentrations diluted three times, with each concentration having two replicates. The IC_{50} values were calculated using XLfit software.

4.3. Molecular modeling

Molecular docking was carried out using Molegro Virtual Docker (MVD) [19]. Compound **11a** and the X-ray crystal structure of xanthine dehydrogenase in complex with febuxostat (PDB entry code 1N5X) were imported. Febuxostat was used to define the binding cavity. The docking algorithm was set at maximum iterations of 1500 with a simplex evolution population size of 50 and a minimum of 10 runs. The schematic diagrams of interactions between xanthine dehydrogenase and docked poses were analyzed by SYBYL software package [22].

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