European Journal of Medicinal Chemistry 190 (2020) 112077

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Design, synthesis and biological evaluation of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles as novel xanthine oxidase inhibitors

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ARTICLE INFO

Article history:
Received 23 December 2019
Received in revised form
11 January 2020
Accepted 16 January 2020
Available online 21 January 2020

Keywords:
Hyperuricemia
Xanthine oxidase inhibitor
1,2,4-Oxadiazole
Biological evaluation

ABSTRACT

Xanthine oxidase (XO) has emerged as an important target for the treatment of hyperuricemia and gout. In this study, to obtain novel nonpurine XO inhibitors, a series of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**1a-1u**, **2c**, **2e**, **2h** and **2n**) were designed using a bioisosteric replacement strategy and were synthesized through a five-step procedure with good yields. Thereafter, the *in vitro* XO inhibitory potencies of these compounds were evaluated by spectrophotometry, showing inhibitory profiles in the micromolar/submicromolar range. Particularly, compound **1h** emerged as the strongest XO inhibitor, with an IC_{50} value of 0.36 μM, which was approximately 21-fold more potent than the positive control allopurinol. Additionally, the structure-activity relationships revealed that the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety linked at the 5-position of the indole scaffold was more preferable than the 6-position for the XO inhibitory potency. Enzyme kinetic studies indicated that compound **1h** acted as a mixed-type XO inhibitor. Moreover, molecular modeling studies were performed on compound **1h** to gain insights into its binding modes with XO. The results showed that the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety could interact with Arg880 and Thr1010 in the innermost part of the active pocket through hydrogen bonds, while the cyano group could form hydrogen bonds with Asn768 and Lys771 in the subpocket. Furthermore, the *in vivo* hypouricemic effect of compound **1h** was further investigated in a hyperuricemia rat model induced by potassium oxonate. The results suggested that compound **1h** could effectively reduce serum uric acid levels at an oral dose of 10 mg/kg. Therefore, compound **1h** could be a promising lead compound for the treatment of hyperuricemia and gout.

1. Introduction

Gout is a common and disabling disease that is associated with chronic hyperuricemia [1,2]. The reported prevalence of gout worldwide ranges from 0.1% to 10% and the incidence of gout has been steadily increasing in many parts of the world in recent years [1,3]. Additionally, gout is associated with multiple comorbidities, such as hypertension, diabetes, cardiovascular disease and chronic kidney disease [4]. Xanthine oxidase (XO), a versatile molybdoflavoprotein, is a critical and rate-limiting enzyme that catalyzes the oxidation of hypoxanthine and xanthine to uric acid in humans [5–8]. Thus, XO is considered the most promising target for the treatment of hyperuricemia and gout [6,9].

To date, only one purine-type XO inhibitor, allopurinol (1966), and two nonpurine-type XO inhibitors, febuxostat (2009) and topiroxostat (2013) (Fig. 1), have been approved for the treatment of hyperuricemia and gout [5]. Allopurinol, an analog of hypoxanthine, was the first marketed XO inhibitor that has been clinically used as a drug for the treatment of hyperuricemia and gout for decades [6]. Unfortunately, allopurinol and its active metabolite oxypurinol (Fig. 1) can be converted by hypoxanthine-guanine

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https://doi.org/10.1016/j.ejmech.2020.112077
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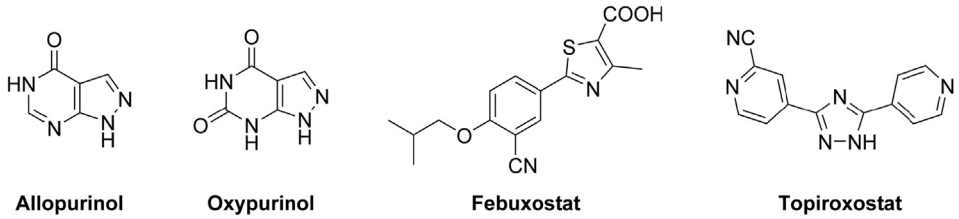


Fig. 1. Chemical structures of selected XO inhibitors.

phosphoribosyltransferase and orotate phosphoribosyltransferase to nucleotide analogs such as allopurinol-1-ribonucleotide and oxypurinol-7-ribonucleotide, which further interfere with normal purine metabolism and pyrimidine synthesis [5,9], and subsequently produce severe side effects such as hypersensitivity, skin rash, gastrointestinal distress, eosinophilia, and renal toxicity [5,6,10]. As a result, various nonpurine-based XO inhibitors with different scaffolds have been extensively investigated, including thiazoles [11–14], pyrazoles [15], isoxazoles [16], imidazoles [17,18], selenazoles [19], furans [20], isocytosines [21–23], pyrimidines [24–27], benzimidazoles [28], 1,2,3-triazole derivatives [29,30], 5-aryl-1*H*-tetrazoles [31], chalcone derivatives [32,33], coumarin derivatives [34–36] and flavonoid derivatives [37,38]. However, febuxostat, a representative nonpurine XO inhibitor, was mandated to have a black box warning by the FDA in February 2019, considering that the cardiovascular mortality of febuxostat was higher than that of allopurinol [39,40]. Therefore, there is an urgent need to develop new nonpurine XO inhibitors for the treatment of hyperuricemia and gout [41].

As a planar acidic heterocycle (pKa = ~6–7), 5-oxo-4,5-dihydro-1,2,4-oxadiazole has been widely adopted by medicinal chemists to design new agents with diverse biological activities, including antifungus, anti-hypertension and anti-inflammation [42–45]. Moreover, it is obvious that the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety could be used as a pyrimidone bioisostere [46–48], considering that allopurinol can be divided into two parts, namely, a pyrimidone and pyrazole. Thus, it is reasonable to use the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety as a pyrimidone bioisostere to design novel allopurinol analogs to obtain new nonpurine XO inhibitors [46–48]. Indole, as a privileged scaffold, could be found in many synthetic and natural products and these products usually possess various biological activities including anti-inflammation, anti-oxidant, anti-hypertension, anti-diabetes, anti-virus and anti-tumor [49–52]. Thus, it is widely used in drug design. Particularly, Song et al. designed a series of 2-indol thiazole derivatives, and the results showed that these compounds exhibited excellent XO inhibitory potency [11,12]. Therefore, it might be a reliable practice to replace the pyrazole ring of allopurinol with an indole scaffold through a bioisosteric replacement strategy.

Accordingly, we connected the pyrimidone and pyrazole rings of allopurinol with a single bond and employed a bioisosteric replacement strategy to replace these two parts of allopurinol with a 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety and an indole scaffold, respectively, to design a series of 1-alkyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**1a-1u**) as novel nonpurine XO inhibitors [15]. Meanwhile, to investigate the influence of the pending 5-oxo-4,5-dihydro-1,2,4-oxadiazole position on the inhibitory potency, the 6-position-substituted compounds 1-alkyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**2c**, **2e**, **2h** and **2n**) were also designed, as is shown in Fig. 2.

In this study, the synthesis, *in vitro* XO inhibitory evaluation and structure-activity relationships of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**1a-1u**, **2c**, **2e**, **2h** and **2n**) are described. In addition, steady-state kinetic analysis and molecular modeling studies were also performed to investigate the inhibitory behaviors of representative compound **1h**. Meanwhile, the hypouricemic effects of compound **1h** were investigated in a potassium oxonate-induced hyperuricemia rat model.

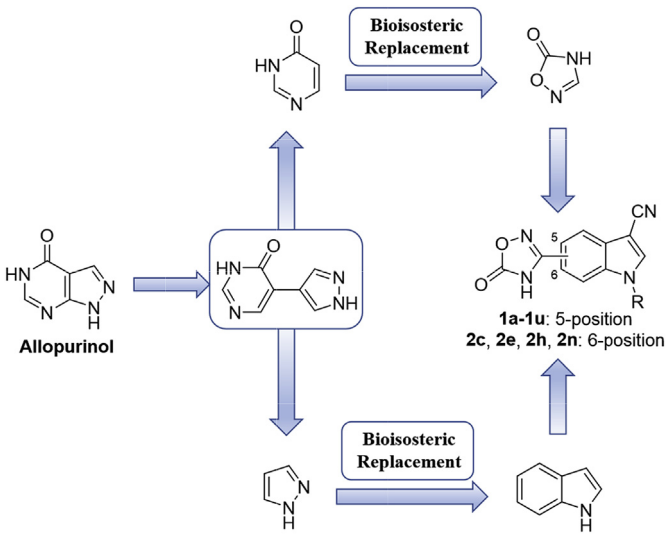
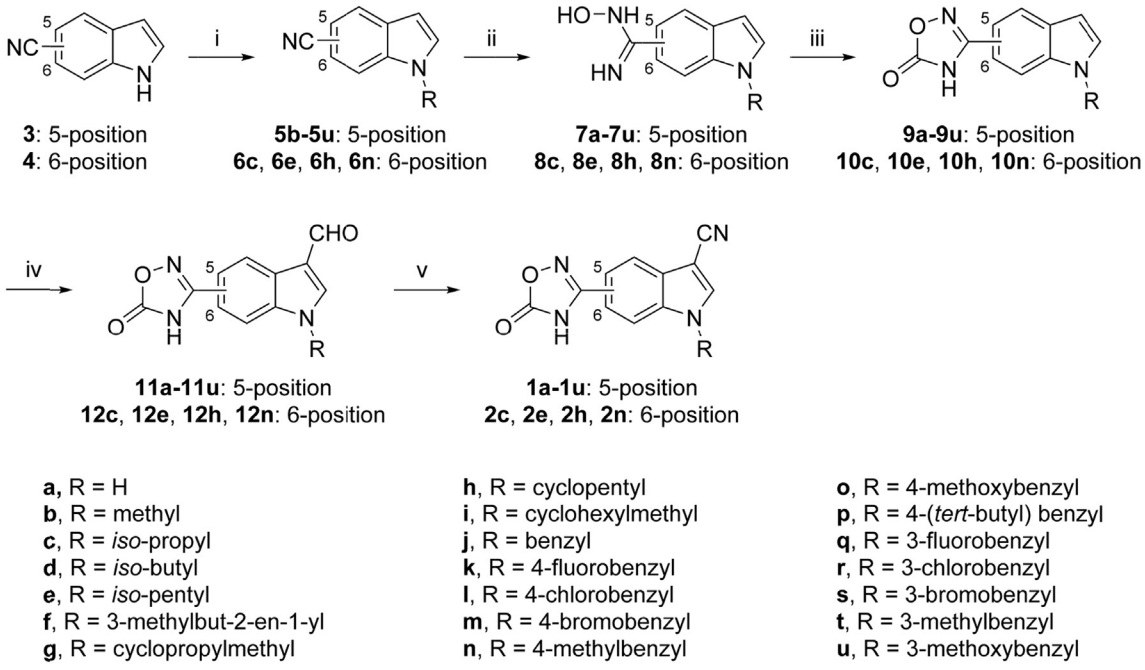


Fig. 2. Design strategy.

2. Results and discussion

2.1. Chemistry

The reaction sequence for the synthesis of the target compounds is shown in Scheme 1. Commercially available 1*H*-indole-5-carbonitrile (**3**) was alkylated with specific alkyl halides in the presence of sodium hydride in dimethylformamide (DMF) to give good yields of 1-alkyl-1*H*-indole-5-carbonitriles (**5b-5u**) [53]. Subsequently, the precursor **3** or intermediates **5b-5u** were reacted with hydroxylamine hydrochloride and sodium carbonate in dimethyl sulfoxide (DMSO) to provide *N*-hydroxy-1-alkyl-1*H*-indole-5-carboximidamides (**7a-7u**), which were further cyclized in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *N*,*N*′-carbonyldiimidazole (CDI) in dichloromethane, furnishing key intermediates 3-(1-alkyl-1*H*-indol-5-yl)-1,2,4-oxadiazol-5(4*H*)-ones (**9a-9u**) with excellent yields [54]. The aldehyde group was introduced into intermediates **9a-9u** under Vilsmeier-Haack conditions to provide 1-alkyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbaldehydes (**11a-11u**) [55]. The corresponding aldehyde group was further converted into a cyano group in the presence of hydroxylamine hydrochloride and sodium formate in formic acid at reflux to afford compounds 1-alkyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**1a-1u**)



Scheme 1. Reagents and conditions: i. RCl or RBr, NaH, KI, DMF, 25–80 °C; ii. $H_2NOH \cdot HCl$, Na_2CO_3, DMSO, 80 °C; iii. CDI, DBU, CH_2Cl_2, 30 °C; iv. $POCl_3$, DMF, 0 °C; v. $H_2NOH \cdot HCl$, HCOONa, NMP, HCOOH, reflux.

in good yields [24]. The 1-alkyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**2c**, **2e**, **2h** and **2n**) were also prepared as previously described using 1*H*-indole-6-carbonitrile (**4**) as a precursor.

2.2. Biological evaluation

2.2.1. Xanthine oxidase inhibitory activity

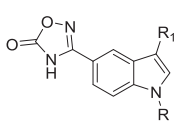
Evaluation of the *in vitro* bovine XO inhibitory potency of compounds **1a-1u**, **2c**, **2e**, **2h**, **2n**, **9c** and **9h** was performed with allopurinol as a reference compound. The results showed that most of the target compounds displayed IC_{50} values ranging between 0.36 μM and 6.56 μM, except **9c** and **9h** (not active, <50% inhibition at 10 μM). In particular, compound **1h** (IC_{50} = 0.36 μM), with a cyclopentyl group at 1-position and a 5-oxo-4,5-dihydro-1,2,4-oxadiazole ring at 5-position of the indole scaffold, exhibited the most active XO inhibitory potency, which was approximately 21-fold more potent than that of allopurinol (IC_{50} = 7.59 μM). Moreover, as exemplified by compounds **9c** and **9h** (<50% inhibition at 10 μM), replacement of the cyano group with a hydrogen atom at 3-position of the indole scaffold resulted in a dramatic loss of potency. This result demonstrated that the cyano group was indispensable for this series of compounds to produce XO inhibitory potency, which was similar to the 2-(indol-5-yl) thiazole derivatives [11].

As shown in Table 1, except for compound **1b** (IC_{50} = 5.41 μM), compounds **1c-1u** (IC_{50} values ranging from 0.36 μM to 3.60 μM), containing a 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety at the 5-position and different alkyl groups at the 1-position of the indole scaffold, were more active than unsubstituted compound **1a** (IC_{50} = 4.13 μM). This finding implied that the hydrophobic group at 1-position of the indole scaffold played a crucial role for these compounds to exhibit XO inhibitory potency, which was consistent with pyrimidones [25]. Specifically, the inhibitory potency of compound **1c** (IC_{50} = 0.41 μM), with an *iso*-propyl group at the 1-position, was approximately 13.2-, 2.7- and 2.5-fold higher than those of compounds **1b**, **1d** and **1e** (IC_{50} = 5.41 μM, 1.09 μM and 1.02 μM, respectively), which indicated that the *iso*-propyl group was favorable for the potency. Interestingly, when the cyclopentyl group was introduced instead of cyclopropylmethyl group in compound **1g** to obtain compound **1h**, the inhibitory potency was increased by approximately 2.4-fold (**1h** vs **1g**, IC_{50} = 0.36 μM and 0.88 μM, respectively), and further expanding the ring to a cyclohexylmethyl group (**1i**, IC_{50} = 2.86 μM) resulted in an approximately 7.9-fold decrease in potency compared with that of compound **1h**, suggesting that the cyclopentyl group was preferable for these saturated cycloalkyl substituted XO inhibitors, which was in accordance with the conclusion from the 1,2,3-triazole derivatives [30].

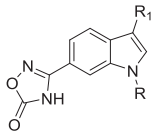
To explore the influence of the benzyl group on inhibitory potency, compounds **1j-1u** with a benzyl or *para*- or *meta*-substituted benzyl group at the 1-position of the indole scaffold were prepared, and the results showed that these compounds also possessed remarkable XO inhibitory potency with IC_{50} values ranging from 1.02 to 3.60 μM. However, by comparing the *meta*-derivatives (**1q**, **1r**, **1s**, **1t** and **1u** IC_{50} = 3.60 μM, 2.93 μM, 2.60 μM, 2.16 μM and 1.70 μM, respectively) to the corresponding *para*-counterparts (**1k**, **1l**, **1m**, **1n** and **1o** IC_{50} = 1.71 μM, 2.40 μM, 2.07 μM, 1.14 μM and 1.02 μM, respectively), the potencies of the *meta*-substituted compounds were weaker than the corresponding *para*-substituted compounds, confirming that, for the halogen (F, Cl and Br), methyl and methoxy groups, the *para* position was preferred for potency.

To investigate the influence of the pending 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety position on potency, compounds **2c**, **2e**, **2h** and **2n** with a 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety substituted at the 6-position of the indole scaffold were prepared. The results showed that the inhibitory potencies of these compounds were largely mitigated (**2c** vs **1c**, **2e** vs **1e**, **2h** vs **1h**, and **2n** vs **1n**; IC_{50} = 1.80 μM vs 0.41 μM, 6.56 μM vs 1.02 μM, 0.82 μM vs 0.36 μM, and 1.84 μM vs 1.14 μM, respectively), which meant that the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety linked at the 5-position was more preferable for the XO inhibitory potency.

Table 1
In vitro XO inhibitory potency of the designed compounds.



1a-1u, 9c, 9h



2c, 2e, 2h, 2n

Compd.	R	R_1	IC_{50} (μM)[a]	Compd.	R	R_1	IC_{50} (μM)[a]
1a	H	CN	4.13 ± 0.15	**1o**	4-methoxybenzyl	CN	1.02 ± 0.01
1b	methyl	CN	5.41 ± 0.09	**1p**	4-(*tert*-butyl) benzyl	CN	1.44 ± 0.07
1c	*iso*-propyl	CN	0.41 ± 0.01	**1q**	3-fluorobenzyl	CN	3.60 ± 0.07
1d	*iso*-butyl	CN	1.09 ± 0.03	**1r**	3-chlorobenzyl	CN	2.93 ± 0.07
1e	*iso*-pentyl	CN	1.02 ± 0.04	**1s**	3-bromobenzyl	CN	2.60 ± 0.07
1f	3-methylbut-2-en-1-yl	CN	1.24 ± 0.03	**1t**	3-methylbenzyl	CN	2.16 ± 0.11
1g	cyclopropylmethyl	CN	0.88 ± 0.03	**1u**	3-methoxybenzyl	CN	1.70 ± 0.05
1h	cyclopentyl	CN	0.36 ± 0.01	**9c**	*iso*-propyl	H	n.a.[b]
1i	cyclohexylmethyl	CN	2.86 ± 0.11	**9h**	cyclopentyl	H	n.a.[b]
1j	benzyl	CN	1.72 ± 0.07	**2c**	*iso*-propyl	CN	1.80 ± 0.06
1k	4-fluorobenzyl	CN	1.71 ± 0.05	**2e**	*iso*-pentyl	CN	6.56 ± 0.32
1l	4-chlorobenzyl	CN	2.40 ± 0.06	**2h**	cyclopentyl	CN	0.82 ± 0.03
1m	4-bromobenzyl	CN	2.07 ± 0.04	**2n**	4-methylbenzyl	CN	1.84 ± 0.06
1n	4-methylbenzyl	CN	1.14 ± 0.03	Allopurinol			7.59 ± 0.21

[a] All values are expressed as the mean ± SD of triplicate determinations.
[b] n.a.: Not active (<50% inhibition at 10 μM).

2.2.2. Enzyme inhibitory kinetics

To explore the mode of action of representative compound **1h,** enzyme kinetic studies were performed. As shown in Fig. 3, Lineweaver-Burk plot analysis revealed that compound **1h** was a mixed-type XO inhibitor, which was different from allopurinol with a competitive inhibition mode [56]. Additionally, the K_m and slope (K_m/V_{max}) were increased but the V_{max} was decreased by increasing the inhibitor concentration from 0 to 1.325 μM. Specifically, the K_m and V_{max} values of compound **1h** were 171.35, 198.38, 203.46, 243.71, and 319.68 μM and 0.189, 0.165, 0.150, 0.107, and 0.041 μM/min, respectively. Moreover, the values of K_i (equilibrium constant for binding with free enzyme) and K_{is} (equilibrium constant for binding with enzyme-substrate complex) were calculated to be 0.138 μM and 0.331 μM, respectively, which confirmed that the inhibitor preferentially bound to the free enzyme rather than to the enzyme-substrate complex [57–59]. Moreover, dose-dependent inhibition of XO by compound **1h** is shown in Fig. 4.

2.2.3. Molecular modeling

To further understand the enzyme-inhibitor interactions, molecular docking of compounds **1h**, **2h** and allopurinol into the binding pocket of the xanthine dehydrogenase (XDH) crystal structure (PDB 1N5X) was performed. The molybdenum-pterin centers of both XDH and XO are identical in terms of binding modes and substrate catalysis [60,61].

As shown in Fig. 5a, compound **1h** was accommodated in the active site through hydrogen bonds and π-π stacking interactions with primary amino acids, including Asn768, Lys771, Arg880, Phe914, Phe1009 and Thr1010, explaining why compound **1h** could produce its XO inhibitory potency. Specifically, the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety interacted with the innermost part of the active pocket via 3 hydrogen bonds, in which the carbonyl group acted as a hydrogen bond acceptor interacting with

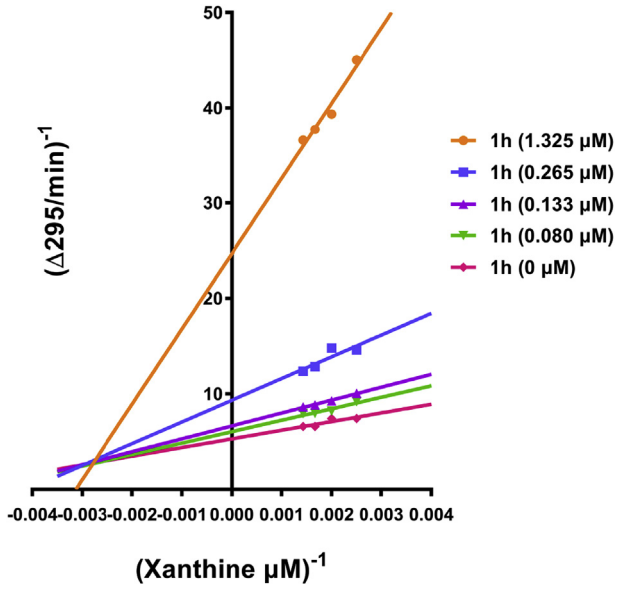


Fig. 3. Lineweaver-Burk plots of XO in the presence of compound **1h**.

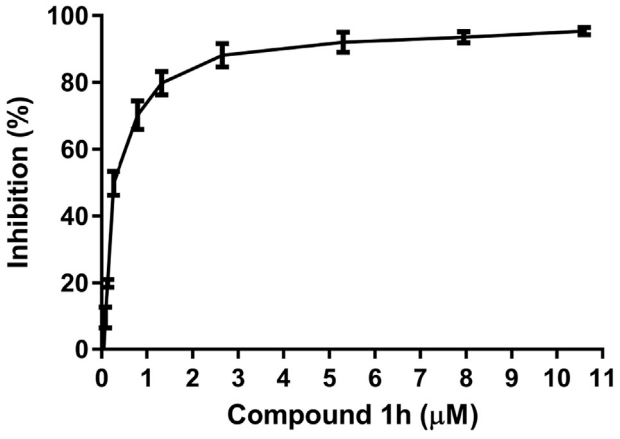


Fig. 4. Dose-dependent inhibition of compound **1h** (means ± SD, n = 3).

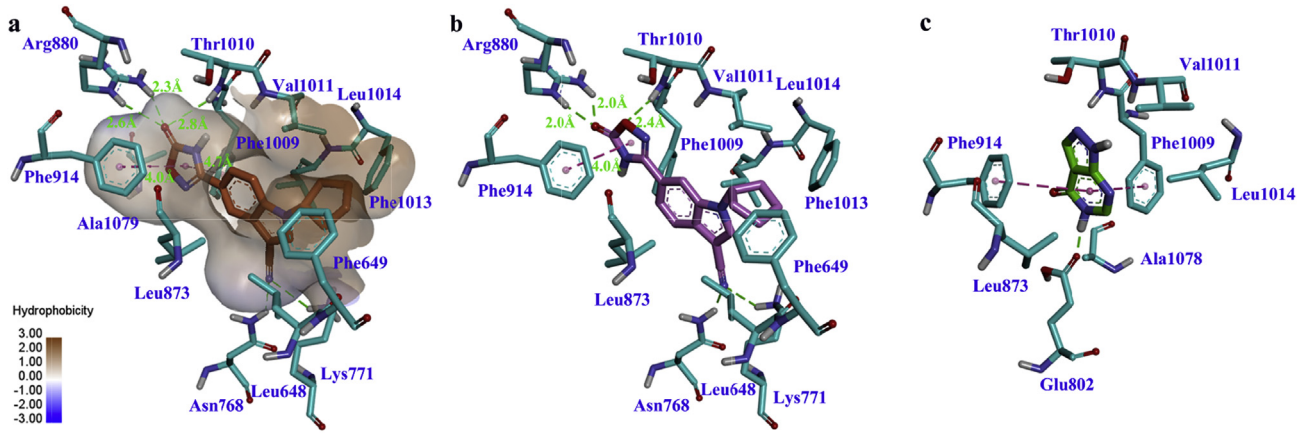


Fig. 5. The binding modes of compounds **1h** and **2h** and allopurinol within the binding pocket of XDH (PDB 1N5X). (a) The interactions of compound **1h** (brown) with the amino acid residues in XDH. (b) The binding mode of compound **2h** (purple) with XDH. (c) The docking pose of allopurinol (green) with XDH. The ligands are depicted as a ball-and-stick model. The amino acid residues in XDH interacting with ligands are shown as cyan lines. The hydrogen bonds and π-π stacking interactions are shown as green and violet dashed lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the amino group of Thr1010 and the guanidine group of Arg880, which was in accordance with 5-aryl-1*H*-tetrazoles [31]. In addition, the 5-oxo-4,5-dihydro-1,2,4-oxadiazole ring was sandwiched between Phe914 and Phe1009 through face-to-face and face-to-edge π-π stacking interactions, which was consistent with allopurinol and pyrano[3,2-d] pyrimidine derivatives [27]. Moreover, the 3-cyano group formed two hydrogen bonds with Asn768 and Lys771 in the subpocket of the active cavity, which was similar to 2-(indol-5-yl) thiazole derivatives and pyrimidones [11,24]. Additionally, several hydrophobic interactions between the cyclopentyl group and Leu648, Phe649, Val1011 and Phe1013 were also observed at the entrance of the active pocket. In contrast, the allopurinol only engaged in one hydrogen bond with Glu802, two π-π stacking interactions with Phe914 and Phe1009 and several hydrophobic interactions with Leu873, Val1011, Leu1014 and Ala1078 (Fig. 5c). Therefore, the binding modes mentioned above could effectively explain the potency difference between compound **1h** and allopurinol; it also emphasized the importance of the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety and 3-cyano group in these compounds to display apparent inhibitory potency. In comparison to compound **1h**, compound **2h** presented similar hydrogen bonds and π-π stacking interactions with the key amino acids including Asn768, Lys771, Arg880, Phe914 and Thr1010 (Fig. 5b). However, the loss of the π-π stacking interaction with Phe1009 may be the main reason for its reduced potency, which could also be observed for other compounds (**2c**, **2e** and **2n**) with a 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety linked at the 6-position of the indole scaffold.

For a deeper understanding of the hydrophobic alkyl groups on the inhibitory potency, molecular docking studies have been also performed on compounds **1b-1e**, **1g** and **1i** at the active pocket. The partial docking scores were calculated by adding the scores of van der Waals force between hydrophobic alkyl groups and amino acids including Leu648, Phe649, Val1011 and Phe1013 at the entrance of the active pocket. As shown in Table 2, the partial docking scores displayed the same tendency with the *in vitro* inhibitory potency of compounds **1b-1e** and **1g-1i**, respectively. And these results provided a reasonable explanation for the observed potencies of these compounds.

2.2.4. Hypouricemic effect in vivo

The *in vivo* hypouricemic effect of compound **1h** (10 mg/kg) was further investigated and compared with the positive control allopurinol (5 mg/kg, Fig. 6). As expected, compared with the blank group, a significant hyperuricemia rat model was established by intraperitoneal injection of potassium oxonate (300 mg/kg) after 1 h of drug administration ($P < 0.0001$). Obviously, compound **1h** showed a reduction of 20.8% in uric acid levels ($P < 0.001$) at 3 h and a reduction of 12.8% in the $AUC_{(\text{uric acid, 1–8 h})}$ ($P < 0.0001$) compared with the model group at an oral dose of 10 mg/kg. Therefore, the results on the hypouricemic effect *in vivo* suggested that compound **1h** could be a promising lead compound for the treatment of hyperuricemia. However, although compound **1h** exhibited a considerable reduction in serum urate levels, it did not show oral efficacy comparable to that of allopurinol. This result could be due to its poor oral bioavailability.

3. Conclusion

In conclusion, using allopurinol as a prototype drug, a novel class of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbonitriles (**1a-1v**, **2c**, **2e**, **2h** and **2n**) were developed as effective XO inhibitors using a bioisosteric replacement strategy. In particular, the *in vitro* biological evaluation results revealed that compound **1h** was the strongest XO inhibitor with an IC_{50} value of 0.36 μM. In addition, the structure-activity relationship analysis revealed that the 3-cyano group was indispensable for these compounds to produce XO inhibitory potency and the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety linked at 5-position rather than 6-position of the indole scaffold was preferable. Additionally, the enzyme kinetic studies indicated that compound **1h** acted as a mixed-type XO inhibitor. The molecular docking results suggested that the 5-oxo-4,5-dihydro-1,2,4-oxadiazole moiety could interact with Arg880 and Thr1010 in the innermost part of the active pocket through 3 hydrogen bonds, while the cyano group could form two hydrogen bonds with Asn768 and Lys771. Moreover, compound **1h** showed an effective hypouricemic effect at an oral dose of 10 mg/kg in a rat model of potassium oxonate-induced hyperuricemia. Accordingly, the results described above strongly suggest that compound **1h** could be a promising lead compound for the treatment of hyperuricemia and gout.

4. Experimental section

4.1. Chemistry

Unless otherwise noted, the solvents and reagents, obtained from commercial companies, were used directly without further

Table 2
Partial docking scores of compounds **1b-1e** and **1g-1i** at the active pocket of XO.

Compounds	R	IC_{50} (μM)	Partial docking score (kcal/mol)
1b	methyl	5.41 ± 0.09	−1.26
1c	*iso*-propyl	0.41 ± 0.01	−8.15
1d	*iso*-butyl	1.09 ± 0.03	−5.06
1e	*iso*-pentyl	1.02 ± 0.04	−4.83
1g	cyclopropylmethyl	0.88 ± 0.03	−9.36
1h	cyclopentyl	0.36 ± 0.01	−11.35
1i	cyclohexylmethyl	2.86 ± 0.11	−6.40

purification. All reactions were run using a Teflon-coated magnetic stir bar at the indicated temperature on the benchtop. Reactions that were run at elevated temperatures were run in an oil bath at the indicated temperature. Reaction progress was monitored by thin-layer chromatography with a 254 nm or 365 nm fluorescence indicator. The purity of the target compounds was determined to be greater than 95% by HPLC. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer or a Bruker 600 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), hept (heptet) or m (multiplet). Low-resolution mass spectrometry (MS) were recorded with an Agilent 1100 ion trap liquid chromatography-mass spectrometer. High-resolution mass spectra (HRMS) data were taken with the Agilent Q-TOF system. Melting points were determined on a YRT-3 apparatus and were uncorrected.

4.1.1. General procedure for the preparation of 1-alkyl-1H-indole-5/6-carbonitriles (5b-5u, 6c, 6e, 6h and 6n)

A solution of **3** (or **4**, 80 mmol) in DMF (80 mL) at 0 °C was treat with sodium hydride (60% dispersion in mineral oil, 90 mmol) portionwise, and the reaction was stirred until the hydrogen was completely evolved. The appropriate alkyl halide (100 mmol) and potassium iodide (8 mmol) were then added to the reaction mixture and the reaction was stirred at 25–80 °C. After completion, the reaction was quenched with caution by the dropwise addition of water, treated with saturated sodium chloride, and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography to afford the title compound.

*4.1.1.1. 1-Methyl-1H-indole-5-carbonitrile (**5b**).* A white crystalline powder; yield 93.6%; M.p 77.1°C–77.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, J = 1.6 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 3.2 Hz, 1H), 7.48 (dd, J = 8.5, 1.6 Hz, 1H), 6.59 (d, J = 3.2 Hz, 1H), 3.84 (s, 3H); ESI-MS m/z 157.05 $[M+H]^+$.

*4.1.1.2. 1-Isopropyl-1H-indole-5-carbonitrile (**5c**).* A yellow oil; yield 81.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09–8.06 (m, 1H), 7.72 (d, J = 2.2 Hz, 1H), 7.70 (d, J = 3.3 Hz, 1H), 7.46 (dd, J = 8.6, 1.6 Hz, 1H), 6.63 (d, J = 3.3 Hz, 1H), 4.84 (hept, J = 6.7 Hz, 1H), 1.46 (d, J = 6.7 Hz, 6H); ESI-MS m/z 185.09 $[M+H]^+$.

*4.1.1.3. 1-Isobutyl-1H-indole-5-carbonitrile (**5d**).* A yellow oil; yield 84.2%; ^{1}H NMR (400 MHz, Chloroform-d) δ 7.96 (dd, J = 1.5, 0.7 Hz, 1H), 7.41 (dd, J = 8.6, 1.5 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 3.2 Hz, 1H), 6.56 (dd, J = 3.2, 0.8 Hz, 1H), 3.93 (d, J = 7.3 Hz, 2H), 2.24–2.11 (m, 1H), 0.92 (d, J = 6.6 Hz, 6H); ESI-MS m/z 199.13 $[M+H]^+$.

*4.1.1.4. 1-Isopentyl-1H-indole-5-carbonitrile (**5e**).* A white crystalline powder; yield 87.1%; M.p 47.8°C–49.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 0.9 Hz, 1H), 7.67 (dt, J = 8.6, 0.9 Hz, 1H), 7.61 (d, J = 3.2 Hz, 1H), 7.48 (dd, J = 8.6, 1.6 Hz, 1H), 6.60 (dd, J = 3.2, 0.8 Hz, 1H), 4.30–4.21 (m, 2H), 1.65 (q, J = 7.0 Hz, 2H), 1.53–1.43 (m, 1H), 0.91 (d, J = 6.6 Hz, 6H); ESI-MS m/z 213.14 $[M+H]^+$.

*4.1.1.5. 1-(3-Methylbut-2-en-1-yl)-1H-indole-5-carbonitrile (**5f**).* A yellow oil; yield 92.4%; ^{1}H NMR (400 MHz, Chloroform-d) δ 7.96 (dd, J = 1.6, 0.7 Hz, 1H), 7.41 (dd, J = 8.6, 1.6 Hz, 1H), 7.38–7.33 (m, 1H), 7.21 (d, J = 3.2 Hz, 1H), 6.55 (dd, J = 3.3, 0.8 Hz, 1H), 5.35 (tdq, J = 6.9, 2.9, 1.4 Hz, 1H), 4.71 (dt, J = 7.0, 1.0 Hz, 2H), 1.83 (d, J = 1.3 Hz, 3H), 1.78 (q, J = 1.3 Hz, 3H); ESI-MS m/z 211.12 $[M+H]^+$.

*4.1.1.6. 1-(Cyclopropylmethyl)-1H-indole-5-carbonitrile (**5g**).* A yellow oil; yield 75.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (dd, J = 1.6, 0.7 Hz, 1H), 7.72 (dd, J = 8.6, 0.8 Hz, 1H), 7.65 (d, J = 3.2 Hz, 1H), 7.47 (dd, J = 8.6, 1.6 Hz, 1H), 6.60 (dd, J = 3.2, 0.8 Hz, 1H), 4.11 (d,

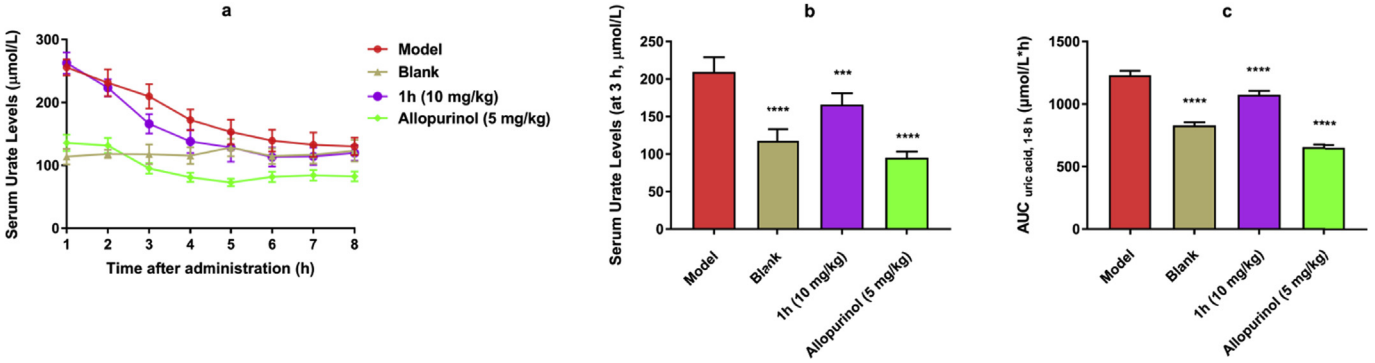


Fig. 6. Anti-hyperuricemic effects of compound **1h** and allopurinol. (a) Time-course changes of serum uric acid levels after 1–8 h of treatment. (b) Serum uric acid levels after 3 h of treatment. (c) The AUC of serum uric acid after 1–8 h of treatment. The data are shown as the mean ± SD. (n = 6). ***P < 0.001, ****P < 0.0001, significantly different from the model group by Student's t-test.

J = 7.0 Hz, 2H), 1.30–1.16 (m, 1H), 0.55–0.47 (m, 2H), 0.44–0.34 (m, 2H); ESI-MS m/z 197.10 $[M+H]^+$.

*4.1.1.7. 1-Cyclopentyl-1H-indole-5-carbonitrile (**5h**).* A yellow oil; yield 57.6%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.95 (t, J = 1.1 Hz, 1H), 7.44 (d, J = 8.6 Hz, 1H), 7.41 (dd, J = 8.6, 1.5 Hz, 1H), 7.31 (d, J = 3.3 Hz, 1H), 6.57 (dd, J = 3.3, 0.6 Hz, 1H), 4.85–4.76 (m, 1H), 2.29–2.19 (m, 2H), 2.01–1.71 (m, 6H); ESI-MS m/z 211.14 $[M+H]^+$.

*4.1.1.8. 1-(Cyclohexylmethyl)-1H-indole-5-carbonitrile (**5i**).* A white crystalline powder; yield 78.2%; M.p 116.2°C–118.1 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (s, 1H), 7.49–7.37 (m, 2H), 7.20 (d, J = 3.2 Hz, 1H), 6.59 (d, J = 3.3 Hz, 1H), 3.99 (d, J = 7.2 Hz, 2H), 1.90–1.80 (m, 1H), 1.77–1.60 (m, 5H), 1.27–1.14 (m, 3H), 1.07–0.97 (m, 2H); ESI-MS m/z 239.17 $[M+H]^+$.

*4.1.1.9. 1-Benzyl-1H-indole-5-carbonitrile (**5j**).* A white crystalline powder; yield 97.3%; M.p 104.5°C–105.6 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (d, J = 1.4 Hz, 1H), 7.42 (dd, J = 8.5, 1.5 Hz, 1H), 7.38–7.25 (m, 5H), 7.12 (dd, J = 7.5, 2.0 Hz, 2H), 6.66 (d, J = 3.2 Hz, 1H), 5.38 (s, 2H); ESI-MS m/z 233.17 $[M+H]^+$.

*4.1.1.10. 1-(4-Fluorobenzyl)-1H-indole-5-carbonitrile (**5k**).* A white crystalline powder; yield 94.1%; M.p 101.9°C–102.5 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (dd, J = 1.6, 0.7 Hz, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.40 (dd, J = 8.6, 1.5 Hz, 1H), 7.28 (dd, J = 8.7, 0.9 Hz, 1H), 7.23 (d, J = 3.2 Hz, 1H), 6.96 (d, J = 8.4 Hz, 2H), 6.64 (dd, J = 3.3, 0.9 Hz, 1H), 5.30 (s, 2H); ESI-MS m/z 251.10 $[M+H]^+$.

*4.1.1.11. 1-(4-Chlorobenzyl)-1H-indole-5-carbonitrile (**5l**).* A white crystalline powder; yield 95.2%; M.p 122.3°C–124.1 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (d, J = 1.4 Hz, 1H), 7.42 (dd, J = 8.6, 1.5 Hz, 1H), 7.33–7.30 (m, 3H), 7.26 (d, J = 3.2 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.67 (dd, J = 3.3, 0.8 Hz, 1H), 5.34 (s, 2H); ESI-MS m/z 267.02 $[M+H]^+$.

*4.1.1.12. 1-(4-Bromobenzyl)-1H-indole-5-carbonitrile (**5m**).* A white crystalline powder; yield 94.4%; M.p 145.4°C–146.7 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (dd, J = 1.6, 0.7 Hz, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.40 (dd, J = 8.6, 1.5 Hz, 1H), 7.28 (dd, J = 8.7, 0.9 Hz, 1H), 7.23 (d, J = 3.2 Hz, 1H), 6.95 (d, J = 8.4 Hz, 2H), 6.64 (dd, J = 3.3, 0.9 Hz, 1H), 5.30 (s, 2H); ESI-MS m/z 310.98, 312.97 $[M+H]^+$.

*4.1.1.13. 1-(4-Methylbenzyl)-1H-indole-5-carbonitrile (**5n**).* A white crystalline powder; yield 92.6%; M.p 69.4°C–70.8 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98–7.97 (m, 1H), 7.38 (dd, J = 8.6, 1.5 Hz, 1H), 7.34–7.30 (m, 1H), 7.23 (d, J = 3.2 Hz, 1H), 7.12 (d, J = 7.8 Hz, 2H), 6.99 (d, J = 7.9 Hz, 2H), 6.61 (dd, J = 3.2, 0.8 Hz, 1H), 5.29 (s, 2H), 2.32 (s, 3H); ESI-MS m/z 247.11 $[M+H]^+$.

*4.1.1.14. 1-(4-Methoxybenzyl)-1H-indole-5-carbonitrile (**5o**).* A white crystalline powder; yield 96.1%; M.p 101.7°C–103.3 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 (dd, J = 1.5, 0.8 Hz, 1H), 7.39 (dd, J = 8.6, 1.5 Hz, 1H), 7.33 (dt, J = 8.5, 0.8 Hz, 1H), 7.22 (d, J = 3.2 Hz, 1H), 7.05 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 6.60 (dd, J = 3.3, 0.9 Hz, 1H), 5.27 (s, 2H), 3.77 (s, 3H); ESI-MS m/z 263.07 $[M+H]^+$.

*4.1.1.15. 1-(4-Tert-butylbenzyl)-1H-indole-5-carbonitrile (**5p**).* A white crystalline powder; yield 85.2%; M.p 111.8°C–113.2 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 (dd, J = 1.6, 0.8 Hz, 1H), 7.39 (dd, J = 8.6, 1.5 Hz, 1H), 7.36–7.31 (m, 3H), 7.24 (d, J = 3.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 2H), 6.61 (dd, J = 3.2, 0.8 Hz, 1H), 5.31 (s, 2H), 1.28 (s, 9H); ESI-MS m/z 289.17 $[M+H]^+$.

*4.1.1.16. 1-(3-Fluorobenzyl)-1H-indole-5-carbonitrile (**5q**).* A white crystalline powder; yield 91.6%; M.p 67.5°C–68.9 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.93 (d, J = 1.4 Hz, 1H), 7.33 (dd, J = 8.6, 1.5 Hz, 1H), 7.23 (d, J = 2.1 Hz, 1H), 7.19–7.16 (m, 2H), 6.91 (td, J = 8.4, 2.5 Hz, 1H), 6.79 (d, J = 7.7 Hz, 1H), 6.68 (dt, J = 9.5, 2.1 Hz, 1H), 6.58 (d, J = 3.2 Hz, 1H), 5.27 (s, 2H); ESI-MS m/z 251.09 $[M+H]^+$.

*4.1.1.17. 1-(3-Chlorobenzyl)-1H-indole-5-carbonitrile (**5r**).* A white crystalline powder; yield 91.5%; M.p 108.2°C–109.5 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (d, J = 1.4 Hz, 1H), 7.43 (dd, J = 8.6, 1.6 Hz, 1H), 7.34–7.26 (m, 4H), 7.13–7.09 (m, 1H), 7.00–6.93 (m, 1H), 6.68 (dd, J = 3.2, 0.8 Hz, 1H), 5.35 (s, 2H); ESI-MS m/z 267.06 $[M+H]^+$.

*4.1.1.18. 1-(3-Bromobenzyl)-1H-indole-5-carbonitrile (**5s**).* A white crystalline powder; yield 90.7%; M.p 117.8°C–118.4 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 (dd, J = 1.6, 0.7 Hz, 1H), 7.45–7.39 (m, 2H), 7.32–7.26 (m, 2H), 7.24 (d, J = 3.2 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.99–6.96 (m, 1H), 6.65 (dd, J = 3.3, 0.9 Hz, 1H), 5.32 (s, 2H); ESI-MS m/z 310.98, 313.00 $[M+H]^+$.

*4.1.1.19. 1-(3-Methylbenzyl)-1H-indole-5-carbonitrile (**5t**).* A yellow oil; yield 87.1%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99–7.96 (m, 1H), 7.40–7.36 (m, 1H), 7.32 (dt, J = 8.6, 0.8 Hz, 1H), 7.24 (d, J = 3.2 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.09 (d, J = 7.7 Hz, 1H), 6.92 (s, 1H), 6.88 (d, J = 7.7 Hz, 1H), 6.61 (dd, J = 3.2, 0.8 Hz, 1H), 5.29 (s, 2H), 2.29 (s, 3H); ESI-MS m/z 247.11 $[M+H]^+$.

*4.1.1.20. 1-(3-Methoxybenzyl)-1H-indole-5-carbonitrile (**5u**).* A white crystalline powder; yield 93.4%; M.p 86.3°C–87.9 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 (dd, J = 1.6, 0.7 Hz, 1H), 7.39 (dd, J = 8.6, 1.5 Hz, 1H), 7.32 (dt, J = 8.6, 0.8 Hz, 1H), 7.25–7.20 (m, 2H), 6.82 (ddd, J = 8.3, 2.6, 0.9 Hz, 1H), 6.67 (ddq, J = 7.6, 1.6, 0.8 Hz, 1H), 6.64–6.59 (m, 2H), 5.31 (s, 2H), 3.73 (s, 3H); ESI-MS m/hept z 263.10 $[M+H]^+$.

*4.1.1.21. 1-Isopropyl-1H-indole-6-carbonitrile (**6c**).* A yellow oil; yield 86.7%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.72 (q, J = 1.0 Hz, 1H), 7.66 (dd, J = 8.2, 0.7 Hz, 1H), 7.42 (d, J = 3.2 Hz, 1H), 7.32 (dd, J = 8.2, 1.3 Hz, 1H), 6.58 (dd, J = 3.2, 0.8 Hz, 1H), 4.68 (hept, J = 6.7 Hz, 1H), 1.55 (d, J = 6.7 Hz, 6H); ESI-MS m/z 185.11 $[M+H]^+$.

*4.1.1.22. 1-Isopentyl-1H-indole-6-carbonitrile (**6e**).* A yellow oil; yield 88.9%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (d, J = 8.2 Hz, 2H), 7.32 (dd, J = 8.1, 1.4 Hz, 1H), 7.29 (d, J = 3.1 Hz, 1H), 6.55 (dd, J = 3.2, 0.8 Hz, 1H), 4.15 (t, J = 7.5 Hz, 2H), 1.74 (q, J = 7.5 Hz, 2H), 1.66–1.53 (m, 1H), 0.98 (d, J = 6.6 Hz, 6H); ESI-MS m/z 213.12 $[M+H]^+$.

*4.1.1.23. 1-Cyclopentyl-1H-indole-6-carbonitrile (**6h**).* A yellow oil; yield 61.3%; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76–7.73 (m, 1H), 7.65 (d, J = 8.2 Hz, 1H), 7.39 (d, J = 3.2 Hz, 1H), 7.31 (dd, J = 8.2, 1.4 Hz, 1H), 6.56 (d, J = 3.2 Hz, 1H), 4.79 (p, J = 7.0 Hz, 1H), 2.29–2.21 (m, 2H), 1.99–1.78 (m, 6H); ESI-MS m/z 211.15 $[M+H]^+$.

*4.1.1.24. 1-(4-Methylbenzyl)-1H-indole-6-carbonitrile (**6n**).* A white crystalline powder; yield 92.4%; M.p 81.3°C–82.9 °C; ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.67 (dd, J = 8.3, 0.7 Hz, 1H), 7.60 (dd, J = 1.4, 0.8 Hz, 1H), 7.34–7.30 (m, 2H), 7.13 (d, J = 7.7 Hz, 2H), 7.00 (d, J = 8.0 Hz, 2H), 6.60 (dd, J = 3.1, 0.9 Hz, 1H), 5.29 (s, 2H), 2.33 (s, 3H); ESI-MS m/z 247.10 $[M+H]^+$.

4.1.2. General procedure for the preparation of N-hydroxy-1-alkyl-1H-indole-5/6-carboximidamides (7a-7u, 8c, 8e, 8h and 8n)

1-Alkyl-1*H*-indole-5/6-carbonitrile (**3**, **5b-5u**, **6c**, **6e**, **6h** and **6n**, 60 mmol), sodium carbonate (120 mmol) and hydroxylamine hydrochloride (72 mmol) were suspended in 60 mL of DMSO. The reaction mixture was stirred at 40 °C for 30 min and then stirred at 80 °C for 2–3 h. After completion, monitored by thin-layer chromatography, the solvent was cooled to room temperature and poured into cold water with vigorous stirring. The resulting precipitate was filtered, washed with water and dried to obtain the corresponding title compound, which was analytically pure and used without further purification.

*4.1.2.1. N-Hydroxy-1H-indole-5-carboximidamide (**7a**).* A white crystalline powder; yield 85.9%; M.p 143.6°C–145.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.37 (s, 1H), 7.87 (d, J = 1.6 Hz, 1H), 7.47 (dd, J = 8.6, 1.7 Hz, 1H), 7.38–7.33 (m, 2H), 6.46 (t, J = 2.7 Hz, 1H), 5.69 (s, 2H); ESI-MS m/z 176.06 $[M+H]^+$.

*4.1.2.2. N-Hydroxy-1-methyl-1H-indole-5-carboximidamide (**7b**).* A white crystalline powder; yield 71.2%; M.p 169.2°C–171.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.85 (d, J = 1.7 Hz, 1H), 7.52 (dd, J = 8.6, 1.7 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.33 (d, J = 3.1 Hz, 1H), 6.44 (dd, J = 3.1, 0.9 Hz, 1H), 5.72 (s, 2H), 3.79 (s, 3H); ESI-MS m/z 190.08 $[M+H]^+$.

*4.1.2.3. N-Hydroxy-1-isopropyl-1H-indole-5-carboximidamide (**7c**).* A white crystalline powder; yield 89.4%; M.p 126.3°C–129.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 7.86–7.83 (m, 1H), 7.51–7.45 (m, 3H), 6.47 (d, J = 3.2 Hz, 1H), 5.68 (s, 2H), 4.75 (hept, J = 6.6 Hz, 1H), 1.45 (d, J = 6.6 Hz, 6H); ESI-MS m/z 218.10 $[M+H]^+$.

*4.1.2.4. N-Hydroxy-1-isobutyl-1H-indole-5-carboximidamide (**7d**).* A white crystalline powder; yield 86.4%; M.p 121.4°C–122.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.84 (dd, J = 1.6, 0.7 Hz, 1H), 7.48 (dd, J = 8.7, 1.6 Hz, 1H), 7.44 (dt, J = 8.8, 0.8 Hz, 1H), 7.35 (d, J = 3.1 Hz, 1H), 6.45 (dd, J = 3.1, 0.7 Hz, 1H), 5.69 (s, 2H), 3.97 (d, J = 7.3 Hz, 2H), 2.20–2.01 (m, 1H), 0.84 (d, J = 6.7 Hz, 6H); ESI-MS m/z 232.11 $[M+H]^+$.

*4.1.2.5. N-Hydroxy-1-isopentyl-1H-indole-5-carboximidamide (**7e**).* A white crystalline powder; yield 91.4%; M.p 139.8°C–141.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 7.84 (d, J = 1.6 Hz, 1H), 7.50 (dd, J = 8.7, 1.7 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.39 (d, J = 3.1 Hz, 1H), 6.44 (dd, J = 3.1, 0.8 Hz, 1H), 4.18 (t, J = 7.3 Hz, 2H), 1.68–1.60 (m, 2H), 1.55–1.42 (m, 1H), 0.91 (d, J = 6.6 Hz, 6H); ESI-MS m/z 284.5 $[M+K]^+$.

*4.1.2.6. N-Hydroxy-1-(3-methylbut-2-en-1-yl)-1H-indole-5-carboximidamide (**7f**).* A white crystalline powder; yield 79.1%; M.p 144.1°C–145.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.85 (dd, J = 1.7, 0.6 Hz, 1H), 7.49 (dd, J = 8.6, 1.6 Hz, 1H), 7.37 (dt, J = 8.7, 0.8 Hz, 1H), 7.33 (d, J = 3.1 Hz, 1H), 6.44 (dd, J = 3.1, 0.8 Hz, 1H), 5.68 (s, 2H), 5.35–5.30 (m, 1H), 4.79–4.73 (m, 2H), 1.81 (d, J = 1.3 Hz, 3H), 1.71 (d, J = 1.3 Hz, 3H); ESI-MS m/z 244.13 $[M+H]^+$.

*4.1.2.7. 1-(Cyclopropylmethyl)-N-hydroxy-1H-indole-5-carboximidamide (**7g**).* A white crystalline powder; yield 86.7%; M.p 162.1°C–163.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.85 (t, J = 1.1 Hz, 1H), 7.51–7.45 (m, 2H), 7.44 (d, J = 3.1 Hz, 1H), 6.45 (d, J = 3.1 Hz, 1H), 5.69 (s, 2H), 4.04 (d, J = 6.9 Hz, 2H), 1.18–1.28 (m, 1H), 0.53–0.47 (m, 2H), 0.40–0.34 (m, 2H); ESI-MS m/z 230.12 $[M+H]^+$.

*4.1.2.8. 1-Cyclopentyl-N-hydroxy-1H-indole-5-carboximidamide (**7h**).* A white crystalline powder; yield 89.3%; M.p 152.3°C–154.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.85–7.83 (m, 1H), 7.51–7.47 (m, 2H), 7.45 (d, J = 3.2 Hz, 1H), 6.46 (d, J = 3.2 Hz, 1H), 5.68 (s, 2H), 4.88 (tt, J = 12.9, 5.6 Hz, 1H), 2.21–2.09 (m, 2H), 1.91–1.79 (m, 4H), 1.76–1.67 (m, 2H); ESI-MS m/z 244.14 $[M+H]^+$.

*4.1.2.9. 1-(Cyclohexylmethyl)-N-hydroxy-1H-indole-5-carboximidamide (**7i**).* A white crystalline powder; yield 83.1%; M.p 192.4°C–194.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 7.84 (d, J = 1.5 Hz, 1H), 7.48 (dd, J = 8.6, 1.6 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.33 (d, J = 3.1 Hz, 1H), 6.44 (d, J = 3.1 Hz, 1H), 5.70 (s, 2H), 4.00 (d, J = 7.2 Hz, 2H), 1.83–1.72 (m, 1H), 1.70–1.55 (m, 3H), 1.53–1.40 (m, 2H), 1.14–1.10 (m, 3H), 1.01–0.92 (m, 2H); ESI-MS m/z 272.15 $[M+H]^+$.

*4.1.2.10. 1-Benzyl-N-hydroxy-1H-indole-5-carboximidamide (**7j**).* A white crystalline powder; yield 86.4%; M.p 158.2°C–159.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.86 (d, J = 1.5 Hz, 1H), 7.52 (d, J = 3.1 Hz, 1H), 7.45 (dd, J = 8.7, 1.6 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.30 (dd, J = 8.1, 6.5 Hz, 2H), 7.26–7.21 (m, 1H), 7.21–7.15 (m, 2H), 6.51 (d, J = 3.1 Hz, 1H), 5.70 (s, 2H), 5.42 (s, 2H); ESI-MS m/z 266.09 $[M+H]^+$.

*4.1.2.11. 1-(4-Fluorobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7k**).* A white crystalline powder; yield 92.6%; M.p 184.5°C–186.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.86 (dd, J = 1.6, 0.7 Hz, 1H), 7.52 (d, J = 3.1 Hz, 1H), 7.46 (dd, J = 8.7, 1.6 Hz, 1H), 7.44–7.41 (m, 1H), 7.28–7.20 (m, 2H), 7.17–7.09 (m, 2H), 6.51 (dd, J = 3.2, 0.7 Hz, 1H), 5.70 (s, 2H), 5.41 (s, 2H); ESI-MS m/z 284.07 $[M+H]^+$.

*4.1.2.12. 1-(4-Chlorobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7l**).* A white crystalline powder; yield 89.3%; M.p 170.9°C–174.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 7.87 (d, J = 1.6 Hz, 1H), 7.52 (d, J = 3.2 Hz, 1H), 7.46 (dd, J = 8.6, 1.7 Hz, 1H), 7.41 (s, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.52 (dd, J = 3.1, 0.7 Hz, 1H), 5.71 (s, 2H), 5.43 (s, 2H); ESI-MS m/z 300.07 $[M+H]^+$.

*4.1.2.13. 1-(4-Bromobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7m**).* A white crystalline powder; yield 89.3%; M.p 195.5°C–197.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.88 (d, J = 1.4 Hz, 1H), 7.54–7.45 (m, 4H), 7.39 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.1 Hz, 2H), 6.53 (d, J = 3.1 Hz, 1H), 5.72 (s, 2H), 5.41 (s, 2H); ESI-MS m/z 344.05, 346.05 $[M+H]^+$.

*4.1.2.14. N-Hydroxy-1-(4-methylbenzyl)-1H-indole-5-carboximidamide (**7n**).* A white crystalline powder; yield 89.3%; M.p 164.7°C–166.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.49 (d, J = 3.1 Hz, 1H), 7.45 (dd, J = 8.6, 1.6 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.09 (s, 4H), 6.49 (dd, J = 3.1, 0.7 Hz, 1H), 5.70 (s, 2H), 5.36 (s, 2H), 2.23 (s, 3H); ESI-MS m/z 280.12 $[M+H]^+$.

*4.1.2.15. N-Hydroxy-1-(4-methoxybenzyl)-1H-indole-5-carboximidamide (**7o**).* A white crystalline powder; yield 79.1%; M.p 163.9°C–165.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.49 (d, J = 3.1 Hz, 1H), 7.48–7.39 (m, 2H), 7.17 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.48 (d, J = 3.1 Hz, 1H), 5.70 (s, 2H), 5.33 (s, 2H), 3.69 (s, 3H); ESI-MS m/z 296.11 $[M+H]^+$.

*4.1.2.16. 1-(4-Tert-butylbenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7p**).* A white crystalline powder; yield 82.4%;

M.p 112.4°C–113.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.86 (d, J = 1.5 Hz, 1H), 7.51 (d, J = 3.1 Hz, 1H), 7.49–7.38 (m, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 6.50 (d, J = 3.1 Hz, 1H), 5.70 (s, 2H), 5.37 (s, 2H), 1.22 (s, 9H); ESI-MS m/z 322.19 $[M+H]^+$.

*4.1.2.17. 1-(3-Fluorobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7q**).* A white crystalline powder; yield 95.6%; M.p 188.7°C–190.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 7.87 (d, J = 1.5 Hz, 1H), 7.54 (d, J = 3.1 Hz, 1H), 7.47 (dd, J = 8.7, 1.6 Hz, 1H), 7.42 (d, J = 8.7 Hz, 1H), 7.34 (td, J = 8.1, 6.1 Hz, 1H), 7.07 (td, J = 8.5, 2.4 Hz, 1H), 7.02–6.97 (m, 2H), 6.53 (d, J = 3.1 Hz, 1H), 5.71 (s, 2H), 5.45 (s, 2H); ESI-MS m/z 284.07 $[M+H]^+$.

*4.1.2.18. 1-(3-Chlorobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7r**).* A white crystalline powder; yield 93.1%; M.p 181.3°C–183.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 7.88 (d, J = 1.5 Hz, 1H), 7.55 (d, J = 3.1 Hz, 1H), 7.47 (dd, J = 8.6, 1.6 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.36–7.30 (m, 2H), 7.24 (d, J = 2.0 Hz, 1H), 7.13 (dt, J = 6.8, 1.9 Hz, 1H), 6.54 (d, J = 3.1 Hz, 1H), 5.72 (s, 2H), 5.45 (s, 2H); ESI-MS m/z 300.06 $[M+H]^+$.

*4.1.2.19. 1-(3-Bromobenzyl)-N-hydroxy-1H-indole-5-carboximidamide (**7s**).* A white crystalline powder; yield 87.9%; M.p 186.6°C–188.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.88 (d, J = 1.5 Hz, 1H), 7.55 (d, J = 3.1 Hz, 1H), 7.50–7.41 (m, 3H), 7.39 (d, J = 1.7 Hz, 1H), 7.26 (t, J = 7.8 Hz, 1H), 7.17 (d, J = 7.7 Hz, 1H), 6.54 (d, J = 3.1 Hz, 1H), 5.73 (s, 2H), 5.44 (s, 2H); ESI-MS m/z 344.05, 346.04 $[M+H]^+$.

*4.1.2.20. N-Hydroxy-1-(3-methylbenzyl)-1H-indole-5-carboximidamide (**7t**).* A white crystalline powder; yield 91.3%; M.p 199.5°C–202.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 7.86 (d, J = 1.5 Hz, 1H), 7.50 (d, J = 3.1 Hz, 1H), 7.46 (dd, J = 8.7, 1.6 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.17 (t, J = 7.5 Hz, 1H), 7.09–7.00 (m, 2H), 6.96 (d, J = 7.6 Hz, 1H), 6.51 (d, J = 3.1 Hz, 1H), 5.71 (s, 2H), 5.37 (s, 2H), 2.23 (s, 3H); ESI-MS m/z 280.11 $[M+H]^+$.

*4.1.2.21. N-Hydroxy-1-(3-methoxybenzyl)-1H-indole-5-carboximidamide (**7u**).* A white crystalline powder; yield 90.0%; M.p 147.6°C–150.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 7.90–7.81 (m, 1H), 7.51 (d, J = 3.1 Hz, 1H), 7.47 (dd, J = 8.7, 1.6 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.20 (t, J = 7.9 Hz, 1H), 6.80 (dd, J = 8.2, 2.6 Hz, 1H), 6.77 (d, J = 1.8 Hz, 1H), 6.72 (dd, J = 7.6, 1.5 Hz, 1H), 6.51 (d, J = 3.1 Hz, 1H), 5.71 (s, 2H), 5.39 (s, 2H), 3.68 (s, 3H); ESI-MS m/z 296.11 $[M+H]^+$.

*4.1.2.22. N-Hydroxy-1-isopropyl-1H-indole-6-carboximidamide (**8c**).* A yellow oil; yield 92.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.82 (d, J = 1.4 Hz, 1H), 7.51 (d, J = 3.2 Hz, 1H), 7.51–7.46 (m, 1H), 7.40 (dd, J = 8.3, 1.4 Hz, 1H), 6.45 (dd, J = 3.2, 0.7 Hz, 1H), 5.80 (s, 2H), 4.77 (hept, J = 6.7 Hz, 1H), 1.47 (d, J = 6.7 Hz, 6H); ESI-MS m/z 218.10 $[M+H]^+$.

*4.1.2.23. N-Hydroxy-1-isopentyl-1H-indole-6-carboximidamide (**8e**).* A white crystalline powder; yield 83.1%; M.p 108.1°C–109.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 7.76 (s, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.43–7.36 (m, 2H), 6.41 (d, J = 3.0 Hz, 1H), 5.80 (s, 2H), 4.19 (t, J = 7.3 Hz, 2H), 1.67 (q, J = 7.1 Hz, 2H), 1.56–1.46 (m, 1H), 0.93 (d, J = 6.6 Hz, 6H); ESI-MS m/z 246.14 $[M+H]^+$.

*4.1.2.24. 1-Cyclopentyl-N-hydroxy-1H-indole-6-carboximidamide (**8h**).* A white crystalline powder; yield 87.3%; M.p 122.3°C–123.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 7.82 (d, J = 1.3 Hz, 1H), 7.48 (dd, J = 5.7, 2.6 Hz, 2H), 7.40 (dd, J = 8.3, 1.4 Hz, 1H), 6.46–6.42 (m, 1H), 5.79 (s, 2H), 4.96–4.82 (m, 1H), 2.23–2.10 (m, 2H), 1.93–1.81 (m, 4H), 1.76–1.69 (m, 2H); ESI-MS m/z 244.14 $[M+H]^+$.

*4.1.2.25. N-Hydroxy-1-(4-methylbenzyl)-1H-indole-6-carboximidamide (**8n**).* A white crystalline powder; yield 93.1%; M.p 176.1°C–177.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.77 (s, 1H), 7.54–7.48 (m, 2H), 7.39 (dd, J = 8.4, 1.4 Hz, 1H), 7.10 (s, 4H), 6.47 (d, J = 3.1 Hz, 1H), 5.74 (s, 2H), 5.37 (s, 2H), 2.23 (s, 3H); ESI-MS m/z 280.12 $[M+H]^+$.

4.1.3. General procedure for the preparation of 3-(1-alkyl-1H-indol-5/6-yl)-1,2,4-oxadiazol-5(4H)-ones (9a-9u, 10c, 10e, 10h and 10n)

N-Hydroxy-1-alkyl-1*H*-indole-5/6-carboximidamide (**7a-7u**, **8c**, **8e**, **8h** and **8n**, 50 mmol) and DBU (100 mmol) were dissolved in dichloromethane (50 mL) at 30 °C. Thereafter, CDI (55 mmol) was added to the reaction mixture, which was stirred at 30 °C for 2 h. After the completion of the reaction, the solvent was evaporated in vacuo. Ice water was added to the residue, and the pH was brought to 4 with 2 M hydrochloric acid. The resulting precipitate was filtered, dried, washed with water and recrystallized with anhydrous methanol to obtain the corresponding title compound.

*4.1.3.1. 3-(1H-Indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9a**).* A white amorphous powder; yield 80.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 1H), 11.53 (s, 1H), 8.06 (s, 1H), 7.55 (d, J = 1.4 Hz, 2H), 7.53–7.48 (m, 1H), 6.59 (dd, J = 3.1, 1.9 Hz, 1H); ESI-MS m/z 200.03 $[M-H]^-$.

*4.1.3.2. 3-(1-Methyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9b**).* A white amorphous powder; yield 86.3%; M.p 223.8°C–225.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 8.05 (t, J = 1.1 Hz, 1H), 7.66–7.57 (m, 2H), 7.47 (d, J = 3.1 Hz, 1H), 6.58 (d, J = 3.1 Hz, 1H), 3.84 (s, 3H); ESI-MS m/z 216.08 $[M+H]^+$.

*4.1.3.3. 3-(1-Isopropyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9c**).* A white amorphous powder; yield 76.2%; M.p 198.8°C–201.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.05 (d, J = 1.7 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 3.3 Hz, 1H), 7.57 (dd, J = 8.7, 1.8 Hz, 1H), 6.62 (d, J = 3.3 Hz, 1H), 4.82 (hept, J = 6.7 Hz, 1H), 1.47 (d, J = 6.7 Hz, 6H); ESI-MS m/z 242.08 $[M-H]^-$.

*4.1.3.4. 3-(1-Isobutyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9d**).* A white amorphous powder; yield 93.2%; M.p 200.1°C–201.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.58 (dd, J = 8.7, 1.7 Hz, 1H), 7.51 (d, J = 3.1 Hz, 1H), 6.60 (dd, J = 3.1, 0.8 Hz, 1H), 4.04 (d, J = 7.3 Hz, 2H), 2.22–2.05 (m, 1H), 0.85 (d, J = 6.7 Hz, 6H); ESI-MS m/z 256.10 $[M-H]^-$.

*4.1.3.5. 3-(1-Isopentyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9e**).* A white amorphous powder; yield 89.2%; M.p 180.8°C–182.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.06 (s, 1H), 7.66 (d, J = 8.7 Hz, 1H), 7.59 (dd, J = 8.7, 1.7 Hz, 1H), 7.54 (d, J = 3.2 Hz, 1H), 6.60 (d, J = 3.1 Hz, 1H), 4.24 (t, J = 7.4 Hz, 2H), 1.66 (q, J = 7.2 Hz, 2H), 1.49 (hept, J = 6.7 Hz, 1H), 0.92 (d, J = 6.7 Hz, 6H); ESI-MS m/z 270.4 $[M-H]^-$.

*4.1.3.6. 3-[1-(3-Methylbut-2-en-1-yl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9f**).* A white amorphous powder; yield 79.3%; M.p 173.2°C–174.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.05 (s, 1H), 7.64–7.56 (m, 2H), 7.48 (d, J = 3.2 Hz, 1H), 6.59 (d, J = 3.2 Hz, 1H), 5.40–5.30 (m, 1H), 4.82 (d, J = 7.0 Hz, 2H), 1.82 (s, 3H), 1.72 (s, 3H); ESI-MS m/z 268.10 $[M-H]^-$.

*4.1.3.7. 3-[1-(Cyclopropylmethyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9g**).* A white amorphous powder; yield 85.6%; M.p 214.0°C–215.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.05 (d, J = 1.6 Hz, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.58 (dd, J = 8.6, 1.9 Hz, 2H), 6.60 (d, J = 3.1 Hz, 1H), 4.10 (d, J = 7.0 Hz, 2H), 1.30–1.20 (m, 1H), 0.53–0.47 (m, 2H), 0.41–0.37 (m, 2H); ESI-MS m/z 254.09 [M − H]$^-$.

*4.1.3.8. 3-(1-Cyclopentyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9h**).* A white amorphous powder; yield 86.8%; M.p 192.6°C–194.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.05 (d, J = 1.6 Hz, 1H), 7.71 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 3.3 Hz, 1H), 7.58 (dd, J = 8.7, 1.7 Hz, 1H), 6.62 (d, J = 3.2 Hz, 1H), 5.01–4.89 (m, 1H), 2.22–2.12 (m, 2H), 1.93–1.78 (m, 4H), 1.78–1.68 (m, 2H); ESI-MS m/z 268.10 [M − H]$^-$.

*4.1.3.9. 3-[1-(Cyclohexylmethyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9i**).* A white amorphous powder; yield 89.7%; M.p 213.0°C–217.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.05 (d, J = 1.6 Hz, 1H), 7.67 (d, J = 8.7 Hz, 1H), 7.58 (dd, J = 8.7, 1.7 Hz, 1H), 7.48 (d, J = 3.1 Hz, 1H), 6.59 (d, J = 3.1 Hz, 1H), 4.06 (d, J = 7.2 Hz, 2H), 1.80 (ddh, J = 11.0, 7.3, 3.6 Hz, 1H), 1.71–1.54 (m, 3H), 1.53–1.43 (m, 2H), 1.18–1.07 (m, 3H), 0.98 (qd, J = 11.7, 3.4 Hz, 2H); ESI-MS m/z 296.16 [M − H]$^-$.

*4.1.3.10. 3-(1-Benzyl-1H-indol-5-yl)-1,2,4-oxadiazol-5(4H)-one (**9j**).* A white amorphous powder; yield 88.7%; M.p 235.8°C–237.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 7.70–7.60 (m, 2H), 7.55 (dd, J = 8.7, 1.7 Hz, 1H), 7.36–7.17 (m, 5H), 6.66 (d, J = 3.1 Hz, 1H), 5.49 (s, 2H); ESI-MS m/z 290.12 [M − H]$^-$.

*4.1.3.11. 3-[1-(4-Fluorobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9k**).* A white amorphous powder; yield 87.4%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.07 (d, J = 1.6 Hz, 1H), 7.71–7.65 (m, 2H), 7.57 (dd, J = 8.7, 1.7 Hz, 1H), 7.31–7.25 (m, 2H), 7.18–7.11 (m, 2H), 6.66 (dd, J = 3.2, 0.8 Hz, 1H), 5.48 (s, 2H); ESI-MS m/z 308.11 [M − H]$^-$.

*4.1.3.12. 3-[1-(4-Chlorobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9l**).* A white amorphous powder; yield 78.7%; M.p 204.4°C–207.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07–8.01 (m, 1H), 7.64–7.57 (m, 2H), 7.53 (dd, J = 8.6, 1.7 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 6.62 (dd, J = 3.2, 0.8 Hz, 1H), 5.44 (s, 2H); ESI-MS m/z 324.11 [M − H]$^-$.

*4.1.3.13. 3-[1-(4-Bromobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9m**).* A white amorphous powder; yield 79.7%; M.p 247.8°C–249.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09–8.06 (m, 1H), 7.66–7.61 (m, 2H), 7.56 (dd, J = 8.6, 1.7 Hz, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 6.66 (dd, J = 3.2, 0.8 Hz, 1H), 5.47 (s, 2H); ESI-MS m/z 368.07, 370.06 [M − H]$^-$.

*4.1.3.14. 3-[1-(4-Methylbenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9n**).* A white amorphous powder; yield 89.7%; M.p 220.1°C–222.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.08–8.05 (m, 1H), 7.67–7.62 (m, 2H), 7.55 (dd, J = 8.7, 1.7 Hz, 1H), 7.11 (s, 4H), 6.64 (dd, J = 3.2, 0.8 Hz, 1H), 5.42 (s, 2H), 2.24 (s, 3H); ESI-MS m/z 304.13 [M − H]$^-$.

*4.1.3.15. 3-[1-(4-Methoxybenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9o**).* A white amorphous powder; yield 90.4%; M.p 227.9°C–229.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.07–8.04 (m, 1H), 7.67 (dt, J = 8.7, 0.8 Hz, 1H), 7.63 (d, J = 3.1 Hz, 1H), 7.55 (dd, J = 8.7, 1.7 Hz, 1H), 7.20 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 6.63 (dd, J = 3.2, 0.8 Hz, 1H), 5.39 (s, 2H), 3.70 (s, 3H); ESI-MS m/z 320.15 [M − H]$^-$.

*4.1.3.16. 3-[1-(4-Tert-butylbenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9p**).* A white amorphous powder; yield 93.7%; M.p 235.4°C–237.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 7.68 (dt, J = 8.7, 0.8 Hz, 1H), 7.65 (d, J = 3.1 Hz, 1H), 7.56 (dd, J = 8.7, 1.7 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.3 Hz, 2H), 6.64 (dd, J = 3.2, 0.8 Hz, 1H), 5.44 (s, 2H), 1.22 (s, 9H); ESI-MS m/z 346.20 [M − H]$^-$.

*4.1.3.17. 3-[1-(3-Fluorobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9q**).* A white amorphous powder; yield 86.3%; M.p 232.2°C–234.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.08 (d, J = 1.7 Hz, 1H), 7.72–7.63 (m, 2H), 7.57 (dd, J = 8.7, 1.7 Hz, 1H), 7.36 (td, J = 8.0, 6.0 Hz, 1H), 7.15–6.99 (m, 3H), 6.68 (d, J = 3.2 Hz, 1H), 5.52 (s, 2H); ESI-MS m/z 308.11 [M − H]$^-$.

*4.1.3.18. 3-[1-(3-Chlorobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9r**).* A white amorphous powder; yield 79.6%; M.p 247.0°C–249.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.08 (d, J = 1.6 Hz, 1H), 7.72–7.64 (m, 2H), 7.57 (dd, J = 8.7, 1.7 Hz, 1H), 7.39–7.25 (m, 3H), 7.15 (dt, J = 6.8, 1.9 Hz, 1H), 6.68 (dd, J = 3.2, 0.8 Hz, 1H), 5.51 (s, 2H); ESI-MS m/z 324.10 [M − H]$^-$.

*4.1.3.19. 3-[1-(3-Bromobenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9s**).* A white amorphous powder; yield 87.4%; M.p 248.5°C–250.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 8.08 (d, J = 1.6 Hz, 1H), 7.71–7.65 (m, 2H), 7.57 (dd, J = 8.7, 1.7 Hz, 1H), 7.48–7.41 (m, 2H), 7.28 (t, J = 7.8 Hz, 1H), 7.19 (dt, J = 7.7, 1.3 Hz, 1H), 6.68 (dd, J = 3.2, 0.8 Hz, 1H), 5.51 (s, 2H); ESI-MS m/z 368.06, 370.06 [M − H]$^-$.

*4.1.3.20. 3-[1-(3-Methylbenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9t**).* A white amorphous powder; yield 87.8%; M.p 225.2°C–227.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (s, 1H), 8.07 (d, J = 1.6 Hz, 1H), 7.67–7.62 (m, 2H), 7.55 (dd, J = 8.7, 1.6 Hz, 1H), 7.19 (t, J = 7.9 Hz, 1H), 7.07 (d, J = 7.1 Hz, 2H), 6.98 (d, J = 7.6 Hz, 1H), 6.65 (d, J = 3.1 Hz, 1H), 5.44 (s, 2H), 2.24 (s, 3H); ESI-MS m/z 304.12 [M − H]$^-$.

*4.1.3.21. 3-[1-(3-Methoxybenzyl)-1H-indol-5-yl]-1,2,4-oxadiazol-5(4H)-one (**9u**).* A white amorphous powder; yield 78.8%; M.p 204.6°C–206.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.07 (dd, J = 1.8, 0.7 Hz, 1H), 7.68–7.64 (m, 2H), 7.56 (dd, J = 8.7, 1.7 Hz, 1H), 7.22 (t, J = 7.9 Hz, 1H), 6.85–6.78 (m, 2H), 6.75–6.73 (m, 1H), 6.65 (dd, J = 3.2, 0.8 Hz, 1H), 5.45 (s, 2H), 3.70 (s, 3H); ESI-MS m/z 320.16 [M − H]$^-$.

*4.1.3.22. 3-(1-Isopropyl-1H-indol-6-yl)-1,2,4-oxadiazol-5(4H)-one (**10c**).* A white amorphous powder; yield 87.2%; M.p 191.0°C–194.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.03 (s, 1H), 7.73–7.69 (m, 2H), 7.48 (dd, J = 8.2, 1.5 Hz, 1H), 6.58 (d, J = 3.2 Hz, 1H), 4.78 (hept, J = 6.7 Hz, 1H), 1.51 (d, J = 6.7 Hz, 6H); ESI-MS m/z 242.09 [M − H]$^-$.

*4.1.3.23. 3-(1-Isopentyl-1H-indol-6-yl)-1,2,4-oxadiazol-5(4H)-one (**10e**).* A white amorphous powder; yield 94.2%; M.p 179.1°C–183.0 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.86 (s, 1H), 7.96 (s, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 3.1 Hz, 1H), 7.47 (dd, J = 8.2, 1.5 Hz, 1H), 6.54 (d, J = 3.1 Hz, 1H), 4.23 (t, J = 7.5 Hz, 2H), 1.69 (q, J = 7.2 Hz, 2H), 1.55–1.48 (m, 1H), 0.93 (d, J = 6.7 Hz, 6H); ESI-MS m/z 270.13 [M − H]$^-$.

*4.1.3.24. 3-(1-Cyclopentyl-1H-indol-6-yl)-1,2,4-oxadiazol-5(4H)-one (**10h**).* A white amorphous powder; yield 79.8%; M.p

178.4°C–180.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.81 (s, 1H), 8.02 (d, J = 1.3 Hz, 1H), 7.72–7.67 (m, 2H), 7.47 (dd, J = 8.2, 1.5 Hz, 1H), 6.57 (d, J = 3.2 Hz, 1H), 4.88 (p, J = 7.2 Hz, 1H), 2.23–2.16 (m, 2H), 1.92–1.83 (m, 4H), 1.78–1.69 (m, 2H); ESI-MS m/z 268.08 [M − H]$^-$.

*4.1.3.25. 3-[1-(4-Methylbenzyl)-1H-indol-6-yl]-1,2,4-oxadiazol-5(4H)-one (**10n**).* A white amorphous powder; yield 84.7%; M.p 213.2°C–214.8 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.84 (s, 1H), 7.98 (d, J = 1.3 Hz, 1H), 7.75–7.71 (m, 2H), 7.47 (dd, J = 8.3, 1.5 Hz, 1H), 7.16–7.10 (m, 4H), 6.60 (d, J = 3.0 Hz, 1H), 5.42 (s, 2H), 2.24 (s, 3H); ESI-MS m/z 304.13 [M − H]$^-$.

4.1.4. General procedure for the preparation of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehydes (11a-11u, 12c, 12e, 12h and 12n)

A 250 mL three-necked round flask was supplemented with 3-(1-alkyl-1*H*-indol-5/6-yl)-1,2,4-oxadiazol-5(4*H*)-one (**9a-9u**, **10c**, **10e**, **10h** and **10n**, 40 mmol) and anhydrous DMF (50 mL) at 0 °C. Phosphorus oxychloride ($POCl_3$, 17 mL) was then added dropwise via an addition funnel maintaining the temperature below 10 °C. The resulting mixture was stirred for 40 min at 0 °C and then room temperature for an additional 1 h. The reaction was quenched with chilled water, followed by the addition of aqueous sodium hydroxide solution. The precipitate was filtered and washed with water. The filter cake was dried in vacuo in the presence of phosphorus pentoxide to yield the title compound, which was analytically pure and used without further purification.

*4.1.4.1. 5-(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11a**).* A white amorphous powder; yield 91.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 10.00 (s, 1H), 8.92 (s, 1H), 8.64 (d, J = 1.5 Hz, 1H), 8.47 (s, 1H), 7.78–7.69 (m, 2H); ESI-MS m/z 228.04 [M − H]$^-$.

*4.1.4.2. 1-Methyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11b**).* A purple amorphous powder; yield 72.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (s, 1H), 9.95 (s, 1H), 8.63 (t, J = 1.2 Hz, 1H), 8.44 (s, 1H), 7.79 (d, J = 1.2 Hz, 2H), 3.95 (s, 3H); ESI-MS m/z 242.06 [M − H]$^-$.

*4.1.4.3. 1-Isopropyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11c**).* A white amorphous powder; yield 79.2%; M.p 220.7°C–222.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H), 9.98 (s, 1H), 8.66–8.65 (m, 2H), 7.90 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7, 1.8 Hz, 1H), 4.93 (hept, J = 6.6 Hz, 1H), 1.55 (d, J = 6.6 Hz, 6H); ESI-MS m/z 270.09 [M − H]$^-$.

*4.1.4.4. 1-Isobutyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11d**).* A purple amorphous powder; yield 81.3%; M.p 238.0°C–240.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.48 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.77 (dd, J = 8.7, 1.8 Hz, 1H), 4.17 (d, J = 7.4 Hz, 2H), 2.24–2.13 (m, 1H), 0.89 (d, J = 6.6 Hz, 6H); ESI-MS m/z 284.11 [M − H]$^-$.

*4.1.4.5. 1-Isopentyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11e**).* A white amorphous powder; yield 84.6%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H), 9.95 (s, 1H), 8.63 (d, J = 1.6 Hz, 1H), 8.51 (s, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.75 (dd, J = 8.7, 1.8 Hz, 1H), 4.34 (t, J = 7.4 Hz, 2H), 1.73 (q, J = 7.0 Hz, 2H), 1.61–1.47 (m, 1H), 0.94 (d, J = 6.6 Hz, 6H); ESI-MS m/z 298.1205 [M − H]$^-$.

*4.1.4.6. 1-(3-Methylbut-2-en-1-yl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11f**).* A purple amorphous powder; yield 87.3%; M.p 228.1°C–230.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 9.96 (s, 1H), 8.64 (d, J = 1.3 Hz, 1H), 8.45 (s, 1H), 7.77 (d, J = 1.2 Hz, 2H), 5.42 (ddt, J = 8.5, 7.0, 1.4 Hz, 1H), 4.94 (d, J = 7.1 Hz, 2H), 1.85 (d, J = 1.3 Hz, 3H), 1.75 (d, J = 1.4 Hz, 3H); ESI-MS m/z 296.13 [M − H]$^-$.

*4.1.4.7. 1-(Cyclopropylmethyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11g**).* A purple amorphous powder; yield 85.5%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.64 (d, J = 1.6 Hz, 1H), 8.54 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.76 (dd, J = 8.7, 1.7 Hz, 1H), 4.20 (d, J = 7.2 Hz, 2H), 1.40–1.29 (m, 1H), 0.64–0.54 (m, 2H), 0.50–0.43 (m, 2H); ESI-MS m/z 282.10 [M − H]$^-$.

*4.1.4.8. 1-Cyclopentyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11h**).* A purple amorphous powder; yield 80.7%; M.p 209.8°C–212.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 9.96 (s, 1H), 8.64 (d, J = 1.7 Hz, 1H), 8.60 (s, 1H), 7.89 (d, J = 8.7 Hz, 1H), 7.76 (dd, J = 8.7, 1.8 Hz, 1H), 5.03 (p, J = 7.0 Hz, 1H), 2.33–2.19 (m, 2H), 2.01–1.82 (m, 4H), 1.82–1.68 (m, 2H); ESI-MS m/z 296.12 [M − H]$^-$.

*4.1.4.9. 1-(Cyclohexylmethyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11i**).* A purple amorphous powder; yield 80.7%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.47 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.79 (dd, J = 8.7, 1.8 Hz, 1H), 4.20 (d, J = 7.3 Hz, 2H), 1.92–1.80 (m, 1H), 1.73–1.47 (m, 5H), 1.24–0.96 (m, 5H); ESI-MS m/z 324.19 [M − H]$^-$.

*4.1.4.10. 1-Benzyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11j**).* A yellow amorphous powder; yield 81.6%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.00 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.63 (s, 1H), 7.80 (dd, J = 8.8, 0.7 Hz, 1H), 7.75 (dd, J = 8.7, 1.7 Hz, 1H), 7.41–7.25 (m, 5H), 5.61 (s, 2H); ESI-MS m/z 318.14 [M − H]$^-$.

*4.1.4.11. 1-(4-Fluorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11k**).* A white amorphous powder; yield 80.4%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.67 (d, J = 2.3 Hz, 2H), 7.85 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7, 1.8 Hz, 1H), 7.43 (dd, J = 8.5, 5.6 Hz, 2H), 7.20 (t, J = 8.8 Hz, 2H), 5.62 (s, 2H); ESI-MS m/z 336.11 [M − H]$^-$.

*4.1.4.12. 1-(4-Chlorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11l**).* A yellow amorphous powder; yield 81.6%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.67 (d, J = 3.3 Hz, 2H), 7.86–7.74 (m, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.2 Hz, 2H), 5.64 (s, 2H); ESI-MS m/z 352.11 [M − H]$^-$.

*4.1.4.13. 1-(4-Bromobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11m**).* A white amorphous powder; yield 83.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 8.66 (d, J = 1.7 Hz, 1H), 8.65 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.75 (dd, J = 8.7, 1.7 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.2 Hz, 2H), 5.61 (s, 2H); ESI-MS m/z 398.06 [M − H]$^-$.

*4.1.4.14. 1-(4-Methylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11n**).* A yellow amorphous powder; yield 83.8%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.61 (s, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.74 (dd, J = 8.7, 1.7 Hz, 1H), 7.24 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 7.8 Hz, 2H), 5.55 (s, 2H), 2.26 (s, 3H); ESI-MS m/z 332.15 [M − H]$^-$.

*4.1.4.15. 1-(4-Methoxybenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11o**).* A white amorphous powder; yield 83.4%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.64 (d, J = 1.6 Hz, 1H), 8.60 (s, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.75 (dd, J = 8.6, 1.7 Hz, 1H), 7.33 (d, J = 8.2 Hz, 2H), 6.92 (d, J = 8.3 Hz, 2H), 5.51 (s, 2H), 3.72 (s, 3H); ESI-MS m/z 348.17 [M − H]$^-$.

*4.1.4.16. 1-(4-Tert-butylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11p**).* A white amorphous powder; yield 83.8%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (s, 1H), 9.99 (s, 1H), 8.65 (d, J = 1.7 Hz, 1H), 8.63 (s, 1H), 7.85 (d, J = 8.7 Hz, 1H), 7.73 (dd, J = 8.7, 1.8 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.3 Hz, 2H), 5.56 (s, 2H), 1.23 (s, 9H); ESI-MS m/z 374.25 [M − H]$^-$.

*4.1.4.17. 1-(3-Fluorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11q**).* A white amorphous powder; yield 87.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.69–8.66 (m, 2H), 7.84 (d, J = 8.7 Hz, 1H), 7.77 (dd, J = 8.7, 1.7 Hz, 1H), 7.41 (td, J = 8.0, 6.1 Hz, 1H), 7.24 (dt, J = 9.9, 2.1 Hz, 1H), 7.16 (dd, J = 8.4, 2.5 Hz, 2H), 5.65 (s, 2H); ESI-MS m/z 336.12 [M − H]$^-$.

*4.1.4.18. 1-(3-Chlorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11r**).* A white amorphous powder; yield 81.6%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.67 (d, J = 1.6 Hz, 2H), 7.84 (d, J = 8.7 Hz, 1H), 7.77 (dd, J = 8.7, 1.8 Hz, 1H), 7.47 (d, J = 2.1 Hz, 1H), 7.42–7.37 (m, 2H), 7.33–7.24 (m, 1H), 5.64 (s, 2H); ESI-MS m/z 352.12 [M − H]$^-$.

*4.1.4.19. 1-(3-Bromobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11s**).* A white amorphous powder; yield 81.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.71–8.62 (m, 2H), 7.84 (d, J = 8.7 Hz, 1H), 7.77 (dd, J = 8.7, 1.8 Hz, 1H), 7.61 (t, J = 1.4 Hz, 1H), 7.55–7.48 (m, 1H), 7.37–7.28 (m, 2H), 5.63 (s, 2H); ESI-MS m/z 396.09, 398.07 [M − H]$^-$.

*4.1.4.20. 1-(3-Methylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11t**).* A white amorphous powder; yield 81.1%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H), 10.00 (s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.62 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.74 (dd, J = 8.7, 1.8 Hz, 1H), 7.24 (t, J = 7.6 Hz, 1H), 7.17 (s, 1H), 7.11 (d, J = 7.6 Hz, 2H), 5.56 (s, 2H), 2.27 (s, 3H); ESI-MS m/z 332.16 [M − H]$^-$.

*4.1.4.21. 1-(3-Methoxybenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**11u**).* A yellow amorphous powder; yield 81.2%; M.p 249.3°C–251.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 8.66 (d, J = 1.6 Hz, 1H), 8.64 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.76 (dd, J = 8.7, 1.7 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1H), 6.96 (t, J = 2.0 Hz, 1H), 6.87 (td, J = 6.6, 3.3 Hz, 2H), 5.57 (s, 2H), 3.73 (s, 3H); ESI-MS m/z 348.15 [M − H]$^-$.

*4.1.4.22. 1-Isopropyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**12c**).* A yellow amorphous powder; yield 86.2%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.67 (s, 1H), 8.27 (d, J = 1.4 Hz, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.74 (dd, J = 8.3, 1.4 Hz, 1H), 4.89 (hept, J = 6.7 Hz, 1H), 1.58 (d, J = 6.6 Hz, 6H); ESI-MS m/z 270.09 [M − H]$^-$.

*4.1.4.23. 1-Isopentyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**12e**).* A yellow amorphous powder; yield 85.6%; M.p 129.4°C–131.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.55 (s, 1H), 8.26–8.19 (m, 2H), 7.80–7.69 (m, 1H), 4.35 (t, J = 7.5 Hz, 2H), 1.76 (q, J = 7.2 Hz, 2H), 1.63–1.53 (m, 1H), 0.96 (d, J = 6.6 Hz, 6H); ESI-MS m/z 298.12 [M − H]$^-$.

*4.1.4.24. 1-Cyclopentyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**12h**).* A white amorphous powder; yield 87.2%; M.p 180.2°C–182.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.61 (s, 1H), 8.26 (s, 1H), 8.22 (d, J = 8.3 Hz, 1H), 7.77–7.72 (m, 1H), 4.99 (t, J = 7.0 Hz, 1H), 2.28 (h, J = 6.1, 5.5 Hz, 2H), 2.11–1.82 (m, 4H), 1.83–1.68 (m, 2H); ESI-MS m/z 296.10 [M − H]$^-$.

*4.1.4.25. 1-(4-Methylbenzyl)-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbaldehyde (**12n**).* A white amorphous powder; yield 84.3%; M.p 205.3°C–206.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.65 (s, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.17 (d, J = 1.4 Hz, 1H), 7.72 (dd, J = 8.3, 1.4 Hz, 1H), 7.28 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 5.54 (s, 2H), 2.26 (s, 3H); ESI-MS m/z 332.15 [M − H]$^-$.

4.1.5. General procedure for the preparation of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitriles (1a-1u, 2c, 2e, 2h and 2n)

1-Alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1*H*-indole-3-carbaldehyde (**11a-11u**, **12c**, **12e**, **12h** and **12n**, 20 mmol), sodium formate (70 mmol), hydroxylamine hydrochloride (40 mmol), formic acid (30 mL) and *N*-methyl pyrrolidone (NMP, 5 mL) were added to a 250 mL round bottom flask. The reaction mixture was stirred under reflux for 6 h. After completion, the mixture was cooled to ambient temperature and then diluted with water. The precipitate was filtered and washed with water. The crude product was purified by flash column chromatography to afford the target compound.

*4.1.5.1. 5-(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1a**).* A purple amorphous powder; yield 70.9%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 12.55 (s, 1H), 8.40 (d, J = 2.8 Hz, 1H), 8.18 (s, 1H), 7.77–7.69 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.41, 158.22, 137.35, 136.89, 127.16, 121.15, 117.99, 117.23, 116.12, 114.55, 85.90; ESI-HRMS calcd. for $C_{11}H_5N_4O_2$ [M − H]$^-$ 225.0418, found 225.0421.

*4.1.5.2. 1-Methyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1b**).* A white amorphous powder; yield 84.3%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.39 (s, 1H), 8.17 (d, J = 1.5 Hz, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.79 (dd, J = 8.7, 1.6 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 158.13, 140.03, 137.89, 127.43, 121.08, 118.07, 117.47, 115.78, 113.20, 84.83, 34.14; ESI-HRMS calcd. for $C_{12}H_7N_4O_2$ [M − H]$^-$ 239.0574, found 239.0576.

*4.1.5.3. 1-Isopropyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1c**).* A white amorphous powder; yield 88.2%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.62 (s, 1H), 8.18 (d, J = 1.7 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.78 (dd, J = 8.8, 1.7 Hz, 1H), 4.93 (hept, J = 6.6 Hz, 1H), 1.50 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.36, 158.08, 136.76, 136.22, 127.55, 121.03, 118.21, 117.49, 115.89, 113.30, 85.54, 48.99, 22.66; ESI-HRMS calcd. for $C_{14}H_{11}N_4O_2$ [M − H]$^-$ 267.0887, found 267.0895.

*4.1.5.4. 1-Isobutyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1d**).* A white amorphous powder; yield 78.2%; M.p 237.5°C–239.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.93 (s, 1H), 8.46 (s, 1H), 8.19 (d, J = 1.7 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.78 (dd, J = 8.7, 1.7 Hz, 1H), 4.15 (d, J = 7.4 Hz, 2H), 2.20–2.13 (m, 1H), 0.86 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 158.09, 139.56, 137.61, 127.40, 121.10, 118.20, 117.43, 115.79, 113.51,

85.15, 54.06, 29.35, 20.01; ESI-HRMS calcd. for $C_{15}H_{13}N_4O_2$ [M − H]⁻ 281.1044, found 281.1051.

*4.1.5.5. 1-Isopentyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1e**).* A white amorphous powder; yield 79.2%; M.p 194.5°C–196.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.49 (s, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.78 (dd, J = 8.7, 1.6 Hz, 1H), 4.32 (t, J = 7.5 Hz, 2H), 1.70 (q, J = 7.2 Hz, 2H), 1.57–1.44 (m, 1H), 0.92 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.39, 158.11, 139.10, 137.18, 127.56, 121.15, 118.24, 117.49, 115.76, 113.23, 85.21, 45.57, 38.56, 25.64, 22.64; ESI-HRMS calcd. for $C_{16}H_{15}N_4O_2$ [M − H]⁻ 295.1200, found 295.1222.

*4.1.5.6. 1-(3-Methylbut-2-en-1-yl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1f**).* A white amorphous powder; yield 81.3%; M.p 215.8°C–217.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.93 (s, 1H), 8.42 (s, 1H), 8.21–8.17 (m, 1H), 7.84–7.77 (m, 2H), 5.38 (tp, J = 6.5, 1.4 Hz, 1H), 4.92 (d, J = 7.0 Hz, 2H), 1.83 (d, J = 1.3 Hz, 3H), 1.74 (d, J = 1.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.36, 158.09, 138.82, 138.38, 137.07, 127.70, 121.12, 118.99, 118.21, 117.51, 115.75, 113.35, 85.20, 45.18, 25.79, 18.42; ESI-HRMS calcd. for $C_{16}H_{13}N_4O_2$ [M − H]⁻ 293.1044, found 293.1046.

*4.1.5.7. 1-(Cyclopropylmethyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1g**).* A white amorphous powder; yield 78.5%; M.p 227.6°C–229.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.52 (s, 1H), 8.19 (s, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H), 4.19 (d, J = 7.2 Hz, 2H), 1.36–1.26 (m, 1H), 0.58–0.54 (m, 2H), 0.47–0.43 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.38, 158.12, 138.94, 137.33, 127.54, 121.12, 118.16, 117.48, 115.78, 113.36, 85.22, 51.33, 11.63, 4.25; ESI-HRMS calcd. for $C_{15}H_{11}N_4O_2$ [M+H]$^+$ 279.0887, found 279.0891.

*4.1.5.8. 1-Cyclopentyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1h**).* A white amorphous powder; yield 76.7%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.56 (s, 1H), 8.16 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 8.8 Hz, 1H), 7.77 (dd, J = 8.8, 1.6 Hz, 1H), 5.02 (p, J = 7.1 Hz, 1H), 2.27–2.16 (m, 2H), 1.94–1.80 (m, 4H), 1.76–1.67 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 158.07, 137.40, 136.53, 127.66, 121.02, 118.14, 117.53, 115.87, 113.50, 85.51, 58.15, 32.54, 23.92; ESI-HRMS calcd. for $C_{16}H_{13}N_4O_2$ [M − H]⁻ 293.1044, found 293.1053.

*4.1.5.9. 1-(Cyclohexylmethyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1i**).* A white amorphous powder; yield 79.7%; M.p 240.0°C–242.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H), 8.41 (s, 1H), 8.17 (s, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.76 (d, J = 8.7 Hz, 1H), 4.15 (d, J = 7.3 Hz, 2H), 1.81 (m, 1H), 1.72–1.39 (m, 5H), 1.05 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.41, 158.11, 139.56, 137.66, 127.41, 121.11, 118.17, 117.44, 115.75, 113.47, 85.13, 52.93, 38.39, 30.30, 26.21, 25.51; ESI-HRMS calcd. for $C_{18}H_{17}N_4O_2$ [M − H]⁻ 321.1357, found 321.1371.

*4.1.5.10. 1-Benzyl-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1j**).* A white amorphous powder; yield 78.6%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.63 (s, 1H), 8.20 (d, J = 1.5 Hz, 1H), 7.89–7.84 (m, 1H), 7.75 (dd, J = 8.8, 1.7 Hz, 1H), 7.40–7.25 (m, 5H), 5.59 (s, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.42, 158.09, 139.57, 137.21, 136.80, 129.28, 128.44, 127.76, 127.71, 121.38, 118.32, 117.75, 115.61, 113.55, 85.76, 50.57; ESI-HRMS calcd. for $C_{18}H_{11}N_4O_2$ [M − H]⁻ 315.0887, found 315.0899.

*4.1.5.11. 1-(4-Fluorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1k**).* A white amorphous powder; yield 83.4%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.62 (s, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.76 (dd, J = 8.7, 1.7 Hz, 1H), 7.38 (dd, J = 8.6, 5.6 Hz, 2H), 7.17 (t, J = 8.9 Hz, 2H), 5.57 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.88, 157.54, 139.06, 136.63, 135.30, 132.62, 129.21, 128.77, 127.19, 120.95, 117.84, 117.31, 115.05, 113.01, 85.40, 49.29; ESI-HRMS calcd. for $C_{18}H_{10}FN_4O_2$ [M − H]⁻ 333.0793, found 333.0797.

*4.1.5.12. 1-(4-Chlorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1l**).* A white amorphous powder; yield 75.6%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.62 (s, 1H), 8.20 (d, J = 1.6 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.76 (dd, J = 8.8, 1.7 Hz, 1H), 7.41 (d, J = 8.6 Hz, 2H), 7.32 (d, J = 8.6 Hz, 2H), 5.59 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 158.05, 139.57, 137.14, 135.81, 133.13, 129.72, 129.28, 127.70, 121.46, 118.35, 117.82, 115.56, 113.52, 85.91, 49.80; ESI-HRMS calcd. for $C_{18}H_{10}ClN_4O_2$ [M − H]⁻ 349.0497, found 349.0523.

*4.1.5.13. 1-Methyl-1H-indole-5-carbonitrile (**5b**)1-(4-Bromobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1m**).* A white amorphous powder; yield 86.3%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.60 (s, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.75 (dd, J = 8.7, 1.7 Hz, 1H), 7.54 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 8.5 Hz, 2H), 5.56 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 158.02, 139.55, 137.11, 136.21, 132.19, 130.01, 127.69, 121.67, 121.44, 118.34, 117.79, 115.56, 113.48, 85.92, 49.86; ESI-HRMS calcd. for $C_{18}H_{10}BrN_4O_2$ [M − H]⁻ 392.9992, found 393.0020.

*4.1.5.14. 1-(4-Methylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1n**).* A white amorphous powder; yield 81.5%; M.p 239.2°C–241.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.90 (s, 1H), 8.59 (s, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.84 (d, J = 8.7 Hz, 1H), 7.74 (dd, J = 8.7, 1.6 Hz, 1H), 7.20 (d, J = 7.9 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 5.51 (s, 2H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.41, 158.07, 139.47, 137.77, 137.13, 133.75, 129.80, 127.84, 127.71, 121.30, 118.28, 117.69, 115.63, 113.55, 85.64, 50.38, 21.10; ESI-HRMS calcd. for $C_{19}H_{13}N_4O_2$ [M − H]⁻ 329.1044, found 329.1069.

*4.1.5.15. 1-(4-Methoxybenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1o**).* A white amorphous powder; yield 76.4%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.58 (s, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.75 (dd, J = 8.8, 1.7 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 5.48 (s, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.47, 159.48, 158.13, 139.33, 137.06, 129.45, 128.64, 127.74, 121.27, 118.26, 117.70, 115.65, 114.65, 113.56, 85.59, 55.57, 50.12; ESI-HRMS calcd. for $C_{19}H_{13}N_4O_3$ [M − H]⁻ 345.0993, found 345.1018.

*4.1.5.16. 1-(4-Tert-butylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1p**).* A white amorphous powder; yield 86.4%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.62 (s, 1H), 8.23–8.17 (m, 1H), 7.94–7.86 (m, 1H), 7.76 (dd, J = 8.8, 1.7 Hz, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 5.54 (s, 2H), 1.23 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 158.05, 150.92, 139.48, 137.20, 133.86, 127.69, 127.55, 126.02, 121.36, 118.29, 117.68, 115.62, 113.55, 85.71, 50.22, 34.70, 31.48; ESI-HRMS calcd. for $C_{22}H_{19}N_4O_2$ [M − H]⁻ 371.1513, found 371.1542.

*4.1.5.17. 1-(3-Fluorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1q**).* A white amorphous powder; yield 87.3%; M.p 219.3°C–231.7 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.63 (s, 1H), 8.20 (d, J = 1.6 Hz, 1H), 7.88 (d, J = 8.7 Hz,

1H), 7.76 (dd, J = 8.7, 1.7 Hz, 1H), 7.39 (td, J = 8.0, 6.0 Hz, 1H), 7.19 (dt, J = 9.9, 2.0 Hz, 1H), 7.16–7.08 (m, 2H), 5.60 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.86, 157.51, 139.07, 138.72, 136.63, 133.38, 130.70, 127.97, 127.26, 127.17, 125.96, 121.00, 117.86, 117.33, 115.03, 112.94, 85.52, 49.34; ESI-HRMS calcd. for $C_{18}H_{10}FN_4O_2$ [M − H]$^-$ 333.0793, found 333.0798.

*4.1.5.18. 1-(3-Chlorobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1r**).* A white amorphous powder; yield 77.6%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.63 (s, 1H), 8.20 (d, J = 1.7 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.76 (dd, J = 8.7, 1.7 Hz, 1H), 7.44 (d, J = 2.1 Hz, 1H), 7.36 (m, 2H), 7.25–7.20 (m, 1H), 5.59 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.36, 158.02, 139.58, 139.23, 137.14, 133.89, 131.20, 128.48, 127.77, 127.67, 126.47, 121.51, 118.36, 117.83, 115.54, 113.44, 86.03, 49.85; ESI-HRMS calcd. for $C_{18}H_{10}ClN_4O_2$ [M − H]$^-$ 349.0497, found 349.0521.

*4.1.5.19. 1-(3-Bromobenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1s**).* A white amorphous powder; yield 84.3%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.64 (s, 1H), 8.20 (d, J = 1.7 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.77 (dd, J = 8.7, 1.7 Hz, 1H), 7.58 (t, J = 1.8 Hz, 1H), 7.50 (dt, J = 7.9, 1.5 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.26 (dt, J = 7.7, 1.3 Hz, 1H), 5.59 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 158.05, 139.60, 139.49, 137.14, 131.49, 131.39, 130.64, 127.67, 126.85, 122.45, 121.53, 118.37, 117.85, 115.54, 113.48, 86.01, 49.78; ESI-HRMS calcd. for $C_{18}H_{10}BrN_4O_2$ [M − H]$^-$ 392.9992, found 393.0021.

*4.1.5.20. 1-(3-Methylbenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1t**).* A white amorphous powder; yield 87.3%; M.p 230.9°C–234.1 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.61 (s, 1H), 8.19 (d, J = 1.6 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.75 (dd, J = 8.7, 1.7 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.14 (s, 1H), 7.10 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 7.7 Hz, 1H), 5.53 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 158.06, 139.57, 138.55, 137.21, 136.70, 129.19, 129.12, 128.36, 127.69, 124.90, 121.36, 118.31, 117.69, 115.64, 113.56, 85.70, 50.56, 21.41; ESI-HRMS calcd. for $C_{19}H_{13}N_4O_2$ [M − H]$^-$ 329.1044, found 329.1068.

*4.1.5.21. 1-(3-Methoxybenzyl)-5-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**1u**).* A white amorphous powder; yield 76.2%; M.p 207.8°C–209.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 8.61 (s, 1H), 8.19 (d, J = 1.7 Hz, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.75 (dd, J = 8.8, 1.7 Hz, 1H), 7.25 (t, J = 7.9 Hz, 1H), 6.92 (t, J = 2.1 Hz, 1H), 6.86 (dd, J = 8.3, 2.4 Hz, 1H), 6.83–6.80 (m, 1H), 5.54 (s, 2H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.37, 159.98, 158.05, 139.59, 138.27, 137.22, 130.45, 127.67, 121.38, 119.83, 118.31, 117.71, 115.62, 113.85, 113.63, 113.56, 85.73, 55.57, 50.50; ESI-HRMS calcd. for $C_{19}H_{13}N_4O_3$ [M − H]$^-$ 345.0993, found 345.1019.

*4.1.5.22. 1-Isopropyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**2c**).* A white amorphous powder; yield 86.2%; M.p > 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 8.65 (s, 1H), 8.20 (d, J = 1.4 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.70 (dd, J = 8.4, 1.4 Hz, 1H), 4.86 (hept, J = 6.7 Hz, 1H), 1.54 (d, J = 6.7 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.36, 158.22, 137.04, 134.67, 130.23, 120.29, 119.66, 118.53, 115.83, 110.84, 85.08, 49.29, 22.56; ESI-HRMS calcd. for $C_{14}H_{11}N_4O_2$ [M − H]$^-$ 267.0887, found 267.0911.

*4.1.5.23. 1-Isopentyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**2e**).* A white amorphous powder; yield 79.7%; M.p 238.7°C–239.9 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.98 (s, 1H), 8.51 (s, 1H), 8.09 (d, J = 1.3 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.68 (dd, J = 8.4, 1.5 Hz, 1H), 4.29 (t, J = 7.6 Hz, 2H), 1.72 (q, J = 7.2 Hz, 2H), 1.56–1.50 (m, 1H), 1H), 0.93 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 158.15, 139.84, 135.08, 130.17, 120.30, 119.59, 118.63, 115.72, 110.59, 84.77, 45.68, 38.38, 25.74, 22.63; ESI-HRMS calcd. for $C_{16}H_{15}N_4O_2$ [M − H]$^-$ 295.1200, found 295.1223.

*4.1.5.24. 1-Cyclopentyl-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**2h**).* A white amorphous powder; yield 67.7%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.59 (s, 1H), 8.19–8.13 (m, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.4, 1.4 Hz, 1H), 4.94 (p, J = 7.2 Hz, 1H), 2.27–2.22 (m, 2H), 1.98–1.80 (m, 4H), 1.79–1.67 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.42, 158.24, 137.33, 135.38, 130.30, 120.24, 119.73, 118.56, 115.84, 111.01, 85.03, 58.34, 32.40, 23.77; ESI-HRMS calcd. for $C_{16}H_{13}N_4O_2$ [M − H]$^-$ 293.1044, found 293.1068.

*4.1.5.25. 1-(4-Methylbenzyl)-6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitrile (**2n**).* A white amorphous powder; yield 89.5%; M.p > 250 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.99 (s, 1H), 8.65 (s, 1H), 8.13 (d, J = 1.4 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.69 (dd, J = 8.4, 1.4 Hz, 1H), 7.22 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 7.8 Hz, 2H), 5.50 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 158.07, 140.28, 137.81, 135.15, 133.69, 130.31, 129.84, 127.79, 120.42, 119.93, 118.87, 115.60, 110.69, 85.27, 50.36, 21.11; ESI-HRMS calcd. for $C_{19}H_{15}N_4O_2$ [M+H]$^+$ 331.1190, found 331.1182.

4.2. Biological evaluation

4.2.1. Assay for the in vitro XO inhibitory potency

In vitro bovine XO inhibitory potency with allopurinol as a positive control was measured by spectrophotometric determination of uric acid formation at 295 nm at 25 °C, with modifications [62]. The enzyme solution (75 U/L) was prepared by dissolving XO (X4875, Sigma-Aldrich Chemical Co.) in phosphate buffer (0.3 mM Na_2EDTA and 0.1 M sodium pyrophosphate decahydrate, pH 8.3). The substrate solution was prepared by dissolving xanthine (J&K) in a buffer. The tested compounds were diluted with buffer to varied concentrations. The reaction was performed in 96-well plates (COSTAR 3599) containing 200 μL of assay mixture, which contained 40 μL of enzyme solution, 53 μL of varying concentrations of the tested compounds (or buffer for the blank) and 67 μL of buffer. After incubating for 25 min at 25 °C, the reaction was initiated by the addition of a 40 μL xanthine solution (500 μM/l), and the XO inhibitory potency of the tested compounds was measured by the reduction of uric acid production in the first 2 min. The final concentration of DMSO is less than 5%. All the compounds were tested in triplicate with 5 different concentrations. Statistical analyses of data were conducted with Microsoft Excel 2016 (Microsoft Inc., Redmond, USA). The IC_{50} values were calculated using IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA) software.

4.2.2. Enzyme inhibitory kinetics

The mode of action of representative compound **1h** was further investigated using enzyme kinetic studies, which were performed in the same way as the *in vitro* XO assay but by varying the xanthine concentrations to 80, 100, 120 and 140 μM/l. The results were analyzed using a Lineweaver-Burk plot, from which we could calculate the value of K_m, V_{max}, K_i and K_{is} in the presence and absence of inhibitor.

4.2.3. Molecular modeling

Molecular modeling studies on the high-resolution crystal structure of XDH (PDB 1N5X) were carried out with GLIDE (2016, Schrodinger Suite) [63,64]. The enzyme preparation, grid, ligands and docking parameter files were in accordance with our previous

report [25]. All ligands were built within Maestro BUILD and prepared using the LIGPREP module (2016, Schrodinger Suite) [63]. The protein was prepared by adding hydrogen atoms, deleting waters and repairing the missing and terminal residues of polypeptide chains using an OPLS force field [63]. The grid size was set as 25 × 25 × 25 Å^3 around the center of the ligand. The conformation with the highest score and rational conformation was selected for discussion. Additionally, the results of docking were visualized using Discovery Studio Visualizer 2017 software [65].

4.2.4. Hypouricemic effect in vivo

Six-week-old male Sprague-Dawley rats were obtained from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The animals were placed in an animal house for 1 week to adapt to the new experimental environment. During this period, the rats were maintained under a 12:12-h light-dark cycle and had free access to food and water. All the study protocols were approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University (Agreement no. SYPU-IACUC-C2019-9-25-210).

Overnight-fasted rats were randomly divided into four groups of six each as follows: blank group, model group, **1h** group and allopurinol group. Potassium oxonate (300 mg/kg) was intraperitoneally injected into rats to induce hyperuricemia, except for the blank group. One hour after administering potassium oxonate, the rats were intragastrically administered with an equal volume of 0.5% CMC-Na, compound **1h** (15 mg/kg) and allopurinol (5 mg/kg) [21,66]. Approximately 0.5 mL of blood sample was collected via orbital vein bleeding at 1–8 h after drug treatment. After coagulation for 2 h at room temperature, the serum sample was obtained by centrifugation at 3500*g* for 10 min at 4 °C. Serum urate levels were determined with a uric acid assay kit (Nanjing Jiancheng Bioengineering Institute, China) in accordance with the manufacturer's instructions. Statistical analysis was analyzed with Student's *t*-test. The figures were obtained with the GraphPad Prism 7.0 (GraphPad Inc., San Diego, USA) statistical system.

4.2.5. Statistical analysis

Data analysis and image processing were performed using Microsoft Excel 2016 (Microsoft Inc., Redmond, USA), IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Inc., San Diego, USA). All data were presented as mean ± standard deviation (SD). The comparisons between two groups were conducted by Student's *t*-test. $P < 0.05$ was considered a statistically significant difference.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by the National Key Research and Development Program of China (2018YFD0200102).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.112077.

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