Med Chem Res (2015) 24:3334–3349
DOI 10.1007/s00044-015-1382-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Screening of a library of 4-aryl/heteroaryl-4*H*-fused pyrans for xanthine oxidase inhibition: synthesis, biological evaluation and docking studies

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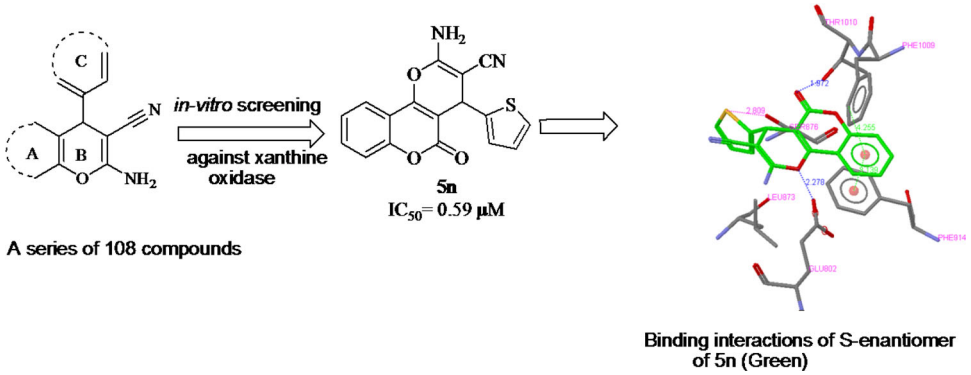
Received: 25 July 2014 / Accepted: 23 May 2015 / Published online: 2 June 2015

Abstract A series of 4-aryl/heteroaryl-4*H*-fused pyrans was synthesized via multicomponent reaction in a microwave synthesizer. All the pyrans were evaluated for in vitro xanthine oxidase inhibition. Structure–activity relationship was also established. Among the series of 108 compounds, Compound **5n** was the most potent displaying remarkable inhibition against the enzyme with an IC_{50} value of 0.59 μM. Enzyme kinetic study was carried out for the compound **5n** to determine the type of inhibition. The study revealed that the compound **5n** was a mixed-type inhibitor. Molecular modelling studies were also performed to figure out the interactions of both the enantiomers of **5n** with the amino acid residues of the enzyme.

Graphical Abstract



Keywords Pyrans · Xanthine oxidase · Inhibition · Molecular modelling studies · Mixed type

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Introduction

Oxidative hydroxylation of hypoxanthine and xanthine catalysed by xanthine oxidase to produce uric acid and reactive oxygen species leads to many diseases such as gout and at least symptoms of diseases such as oxidative

damage to the tissue (Sharma *et al.*, 2014; Stockert *et al.*, 2002; Borges *et al.*, 2002; Hille, 2006). Therefore, the selective inhibition of XO may result in broad-spectrum chemotherapeutic for gout, cancer, inflammation and oxidative damage (Borges *et al.*, 2002; Hille, 2006; Pacher *et al.*, 2006). Allopurinol (Hille, 2006; Pacher *et al.*, 2006), 2-alkyl hypoxanthines (Biagi *et al.*, 2001; Robins *et al.*, 1985), pterin and 6-formylpterin (Oettl and Reibneggar, 1999) represent the class of purine-based xanthine oxidase inhibitors. All these inhibitors have been successfully utilized and have proved their inhibitory potential towards the enzyme. However, these purine-based inhibitors have been reported to be associated with Steven–Johnson syndrome and worsening of renal function induced in some of the patients (Borges *et al.*, 2002; Hille, 2006; Pacher *et al.*, 2006). Keeping in view these side effects, our research group has been actively involved in the design of some non-purine xanthine oxidase inhibitors in the recent past such as azaflavones (Nepali *et al.* 2011a, b), *n*-acetyl pyrazolines (Nepali *et al.* 2011a, b), β-acetamido compounds (Dhiman *et al.*, 2012), naphthopyrans (Sharma *et al.*, 2014) and 4,6-diaryl/heteroarylpyrimidin-2(1*H*)-ones (Shukla *et al.*, 2014).

Polyfunctionalized 4*H*-pyrans have a unique role in medicinal chemistry due to their wide range of biological and pharmacological activities (Elnagdi *et al.*, 1983; Goldmann and Stoltefus, 1991). These compounds have been utilized as anticancer agents, anticoagulants, spasmolytics and antianaphylactics (Andreani and Lapi, 1960; Bonsignore *et al.*, 1993). 4*H*-Pyran derivatives containing heterocyclic rings are extensively used for their pharmacological activities (Green *et al.*, 1995; Sanchez *et al.*, 2012). Fused pyran derivatives also exhibit a wide spectrum of pharmacological activities and biological activities, such as insecticidal (Uher *et al.*, 1994), antiviral and antileishmanial (Perez-Perez *et al.*, 1995; Fan *et al.*, 2010), anticonvulsant and antimicrobial activities (Aytemir *et al.*, 2004). Also, many of them are non-peptide human immunodeficiency virus (HIV) protease inhibitors (Wang *et al.*, 1996; Pochet *et al.*, 1996; Mazumder *et al.*, 1996). Pyrans are also an important structural motif in number of non-purine xanthine oxidase inhibitors (Nepali *et al.* 2011a, b; Star and Marby, 1971; Cos *et al.*, 1998). Coumarins and flavonoids represent the class of fused pyrans as non-purine xanthine oxidase inhibitors (Nepali *et al.* 2011a, b; Cos *et al.*, 1998; Da-Silva *et al.*, 2004; Lin *et al.*, 2002). Both the classes have been extensively explored for their xanthine oxidase inhibitory potential and insights about the structure–activity relationship, and their interactions with the amino acid residues of the enzyme have also been figured out. Recently working on similar lines, our research group synthesized and evaluated a series of naphthopyrans for in vitro xanthine oxidase inhibition in view of some of the potent non-purine xanthine oxidase inhibitors possessing benzopyran skeleton. The potent inhibitory potential of some naphthopyrans was attributed to the interactions of pyran ring as indicated by molecular modelling studies (Sharma *et al.*, 2014).

In continuation of our search for non-purine-based xanthine oxidase inhibitors (Dhiman *et al.*, 2012; Nepali *et al.* 2011a, b; Sharma *et al.*, 2014; Singh *et al.*, 2014; Shukla *et al.*, 2014; Virdi *et al.*, 2014) and motivated by the promising xanthine oxidase inhibitory potential of naphthopyrans, the present study screens a library of fused pyrans in diverse scaffolds for xanthine oxidase inhibition. A library of 4-aryl/heteroaryl-4*H*-fused pyrans was synthesized and evaluated against the enzyme. The type of inhibition and the interactions of the most potent inhibitor with the amino acid residues of the enzyme have also been figured out.

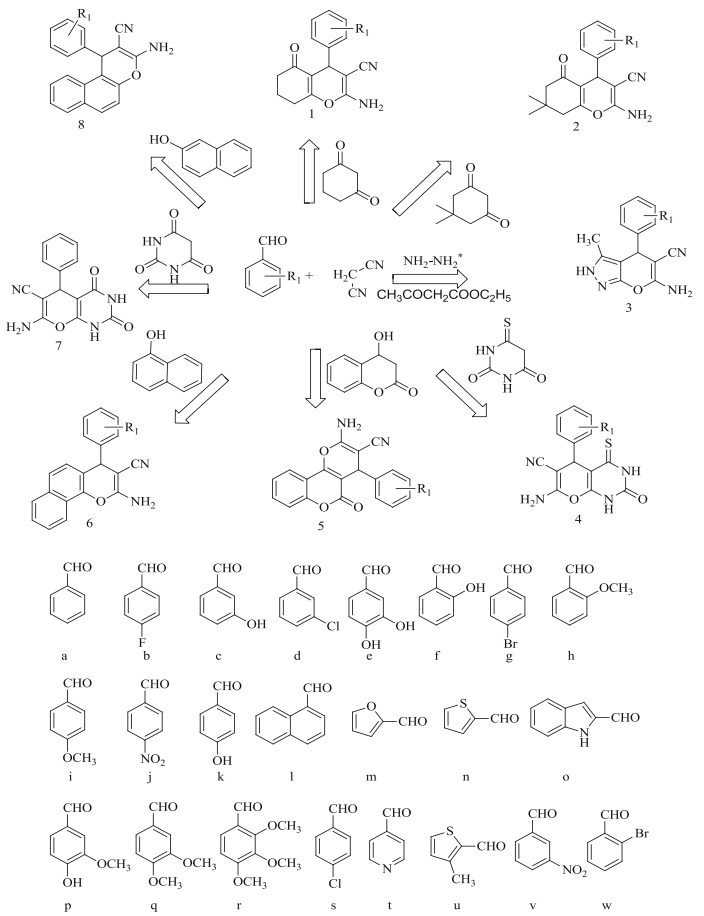
Results and discussion

Synthesis

A library of 4*H*-pyrans was synthesized as shown in Scheme 1. The compounds were synthesized by exposing a mixture of aromatic aldehyde, malononitrile, C–H-activated acidic compound and catalytic amount of DMAP to microwave radiation in a microwave synthesizer operating at 150 °C with the maximum microwave power of 400 W (Scheme 1). The structures of the synthesized compounds were elucidated by ^{1}H NMR and ^{13}C NMR. All spectral data were in accordance with assumed structures.

In vitro xanthine oxidase assay

In vitro screening of the pyrans using bovine milk xanthine oxidase (grade 1, ammonium sulphate suspension) enzymatic assay was performed as described in the literature (Escribano *et al.*, 1988; Takano *et al.*, 2005). Allopurinol (Pacher *et al.*, 2006) was employed as reference inhibitor. The molecules exhibiting % age inhibition of more than 80 % at 50 μM were further tested in triplicate for the xanthine oxidase inhibitory activity to calculate the IC_{50} values. Among a series of 108 compounds, 41 compounds were found to display a % age inhibition of >80 % and were tested at different concentration against xanthine oxidase (Table 1; Fig. 1). Compounds **5m** and **5n** displayed significant inhibitory potential with IC_{50} values, 0.9 and 0.59 μM, respectively (IC_{50} value of allopurinol = 8.29 μM). Figure 2 shows interesting structure–activity relationship for the inhibitory effects against the enzyme. Careful observation of the IC_{50} values of the compounds indicates that nature of Ring A and Ring C remarkably influences the activity. Few generalizations



Scheme 1 Synthesis of 4*H*-pyrans. *C–H-activated acidic compound in case of 3 was prepared from the reaction of hydrazine hydrate and ethyl acetoacetate

* C-H activated acidic compound in case of 3 was prepared from the reaction of hydrazine hydrate and ethyl acetoacetate.

about the structure–activity relationship are as follows: (1) compounds with nitro- and halo-substituted Ring C (**1g**, **1j**, **1s**, **2s**, **3g**, **3j**, **3s**, **4g**, **4j**, **4s**, **5g**, **5j**, **5s**, **6g**, **6j**, **6s**, **7g**, **7j**, **7s**, **8g**, **8j**, **8s**) exhibited significant inhibition, whereas compounds with methoxy- and hydroxy-substituted phenyl rings (**1e**, **1h**, **1i**, **1k**, **1p**, **1q**, **2e**, **2f**, **2h**, **2i**, **2k**, **2q**, **3c**, **3f**, **3h**, **3i**, **3k**, **3q**, **3r**, **4h**, **4i**, **5h**, **5i**, **5k**, **5p**, **5q**, **6e**, **6h**, **6i**, **6k**, **6q**, **7h**, **7i**, **8h**, **8i**) did not qualify for the evaluation at different concentration (i.e. % age inhibition <80). (2) The influence of placement of substitution was also evident from Fig. 1 as compounds with para substituted phenyl rings (halo and nitro groups) were the only one to pass the initial screening at 50 μM by displaying % age inhibition of >80 (**1g**, **1j**, **1s**, **2s**, **3g**, **3j**, **3s**, **4g**, **4j**, **4s**, **5q**, **5j**, **5s**, **6g**, **6j**, **6s**, **7g**, **7j**, **7s**, **8g**, **8j**, **8s**). (3) The compounds with substituted phenyl rings (substitutions other than nitro and halo, Ring C) were even less active than compounds bearing unsubstituted phenyl ring (Ring C) (**1a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a**). (4) Replacement of phenyl rings with naphthyl ring (compare **1a**, **3a**, **5a** with **1l**, **3l**, **5l**) resulted in decline in the inhibitory potential of the compounds as naphthyl-substituted (Ring C) inhibitors displayed % age inhibition of <80 % and did not qualify for evaluation at different concentrations. (5) Replacement of phenyl rings with heteroaryl rings (Ring C) resulted in drastic improvement in the activity (compare **1a**, **2a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a** with **1m**, **1n**, **2n**, **3m**, **3n**, **4n**, **5m**, **5n**, **6n**, **7n**, **8m**, **8n**). (6) Among the heteroaryl-substituted compounds (Ring C), compounds with thiophenyl Ring C were more active than the compound with furanyl ring (Ring C) (compare **1n**, **3n**, **5n**, **8n** with **1m**, **3m**, **5m**, **8m**). This could be attributed to the higher aromatic character of thiophene ring as compared to Furan ring. Overall the preference order for Ring C is as follows: thiophene > furanyl > phenyl with halo (preferable chloro at para position) > phenyl with nitro (para substituted). (7) Ring A also displayed significant influence on the inhibitory potential. Coumarin-substituted (Ring A) compounds were found to be the most active (compare **5a** with **1a**, **2a**, **3a**, **4a**, **6a**, **7a**, **8a**). (8) Compounds with bicyclic ring (Ring A) also displayed a significant inhibitory potential higher than compounds with monocyclic rings (compare **5a**, **6a**, **8a** with **1a**, **2a**, **3a**, **4a**, **7a**). (9) Both 1-naphthyl- and 2-naphthyl-substituted pyrans (Ring A) displayed promising results; however, no competition was observed between the activity profiles of two. Overall the preference order for Ring A is as follows:

Table 1 % age inhibition of the synthesized compounds at 50 μM

Code	% age inhibition (50 μM)	Code	% age inhibition (50 μM)	Code	% age inhibition (50 μM)
1a	81	**3i**	38	**5w**	25
1b	62	**3j**	82	**6a**	87
1d	59	**3k**	45	**6b**	43
1e	42	**3l**	54	**6d**	61
1g	88	**3m**	84	**6e**	68
1h	55	**3n**	89	**6g**	82
1i	56	**3o**	44	**6h**	64
1j	84	**3q**	55	**6i**	41
1k	66	**3r**	52	**6j**	86
1l	42	**3s**	88	**6k**	47
1m	84	**3t**	79	**6n**	88
1n	89	**3u**	74	**6q**	63
1p	46	**4a**	83	**6s**	89
1q	59	**4d**	31	**6v**	35
1s	88	**4g**	84	**7a**	80
1u	43	**4h**	10	**7d**	46
1v	56	**4i**	63	**7g**	85
2a	68	**4j**	85	**7h**	32
2b	75	**4n**	90	**7i**	70
2e	35	**4s**	88	**7j**	81
2f	50	**4u**	37	**7n**	89
2g	73	**4v**	66	**7s**	83
2h	59	**5a**	89	**7u**	68
2i	37	**5b**	75	**7v**	70
2j	58	**5g**	89	**8a**	85
2k	23	**5h**	42	**8b**	33
2n	83	**5i**	37	**8g**	87
2q	63	**5j**	85	**8h**	66
2s	87	**5k**	34	**8i**	51
2v	26	**5l**	49	**8j**	83
3a	83	**5m**	93	**8m**	85
3b	45	**5n**	96	**8n**	87
3c	24	**5o**	66	**8s**	86
3d	62	**5q**	65	**8v**	44
3f	27	**5s**	90		
3g	85	**5p**	52		
3h	48	**5v**	15		

coumarin > 1-naphthyl = 2-naphthyl > pyrazole > cyclohexanedione > thiobarbituric acid > barbituric acid > 5,5-dimethyl cyclohexanedione. Figure 2 represents the structure–activity relationship.

Compound **5n**, the most active of the series with an IC_{50} value 0.59 μM, was further investigated for enzyme kinetics study and molecular modelling studies.

Enzyme kinetic study

Compound **5n** was further investigated for the type of inhibition, and enzyme kinetics study was carried out. The Lineweaver–Burk plot (Fig. 3) revealed that the compound **5n** was mixed-type XO inhibitor. The pattern of graph shows that it is a form of mixed inhibition scenario. The K_m, V_{max} and slope are all affected by concentration of the inhibitor. The inhibitor has increased the K_m and slope (K_m/V_{max}) while decreasing the V_{max}. Moreover, Figure 3 shows that intersecting lines on the graph converge to the left of the *y*-axis and above the *x*-axis which indicates that the value of α (a constant that defines the degree to which inhibitor binding affects the affinity of the enzyme for substrate) is >1. Mixed-type inhibitors are those which are capable of binding to both the free enzyme and the enzyme–substrate complex. However, keeping in view the pattern of intersecting lines on the graph, it can be assumed that the inhibitor preferentially binds to the free enzyme and not the enzyme–substrate complex (Copeland, 2005).

Molecular modelling study

Molecular docking study was performed to get structural insights into the binding behaviour of the potent compound **5n**. A flexible docking study was performed using Gold Software (GOLD 2012). Compound **5n** has a chiral centre; therefore, both *R* and *S* conformations of **5n** were docked. The binding poses with highest fitness score were selected, and their binding interactions were studied.

The docking study reveals that *S*-enantiomer of **5n** fits well in the binding site, while *R*-enantiomer was not able to get in the cavity (Fig. 4). The binding interactions of *S*-enantiomer with highest score were studied. In binding pose, *S*-enantiomer of **5n** fits well in the binding cavity and gets stabilized by various molecular interactions. The chromene ring gets sandwiched in Phe914 and Phe1009 showing "face-to-face" and "edge-to-face" pie-stacking, respectively. The carbonyl group of chromene ring was found to involve in hydrogen bonding with Thr1010. Another hydrogen bonding was observed between Glu802 and oxygen of **5n**. The sulphur of thiophene ring was found to be involved in van der Waals interactions with Ser876. The above interactions provided an insight behind the inhibition of XO by **5n** (Fig. 4).

Conclusion

Allopurinol, a well-known xanthine oxidase inhibitor, is a competitive inhibitor and has been employed as standard for the in vitro and in vivo studies over the years. However, its use has been associated with some complications. The

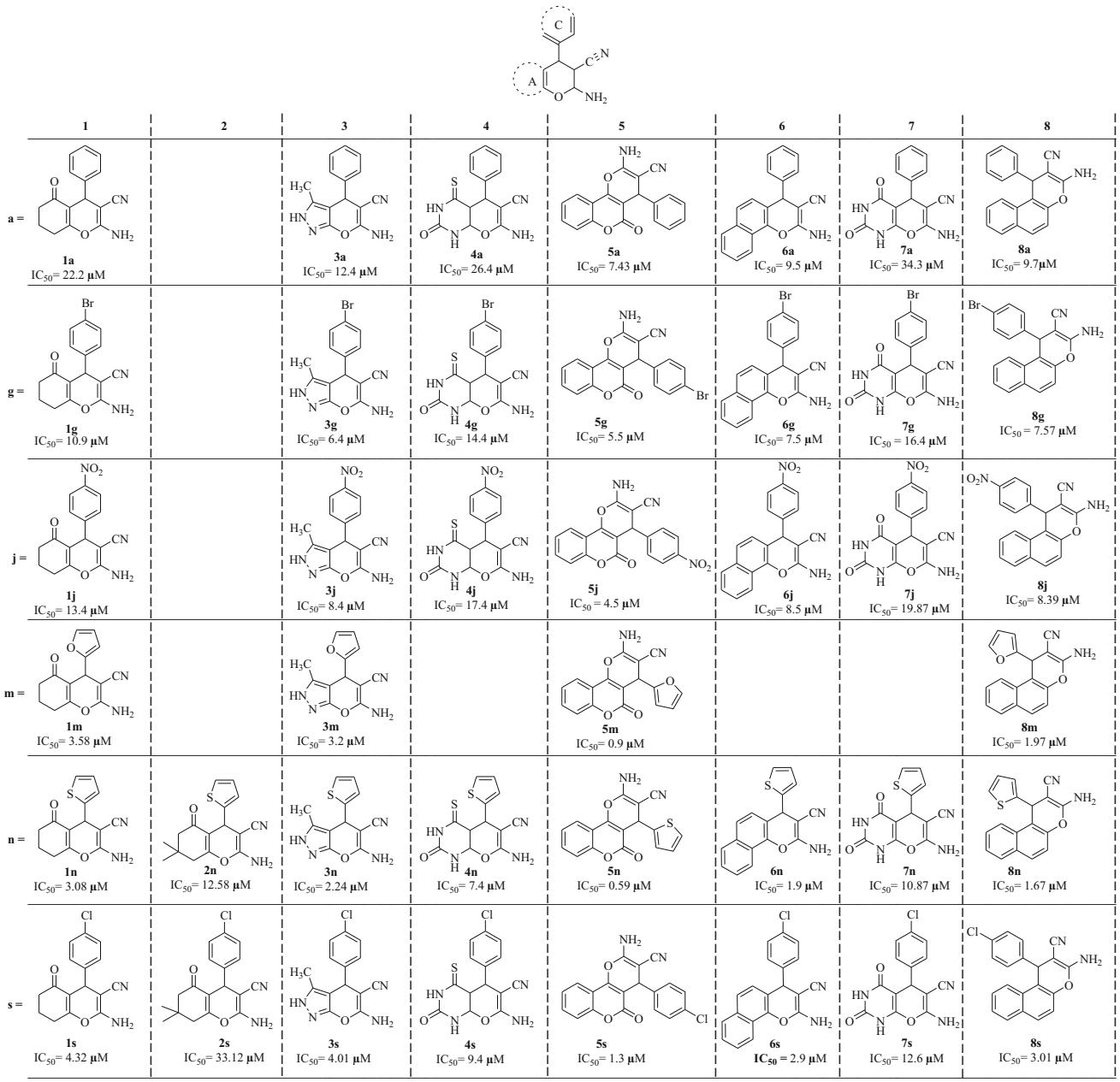


Fig. 1 IC_{50} values of selected compounds (IC_{50} value of allopurinol = 8.29 μM)

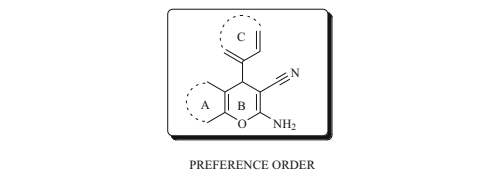


Fig. 2 Structure–activity relationship

competitive inhibitors are basically purine-based structures, and the interactions of purine analogue XO inhibitors with the activities of purine and pyrimidine metabolism enzymes such as guanine deaminase, HGPRT (hypoxanthine–guanine phosphoribosyltransferase), PNP (purine nucleoside phosphorylase), OPRT (orotate phosphoribosyltransferase) and OMPDC (orotidine-5-monophosphate decarboxylase) leading to the hypersensitivity (Steven–Johnson) a syndrome characterized by fever, skin rash, hepatitis, leukocytosis with eosinophilia and worsening renal function induced in some of the patients has basically

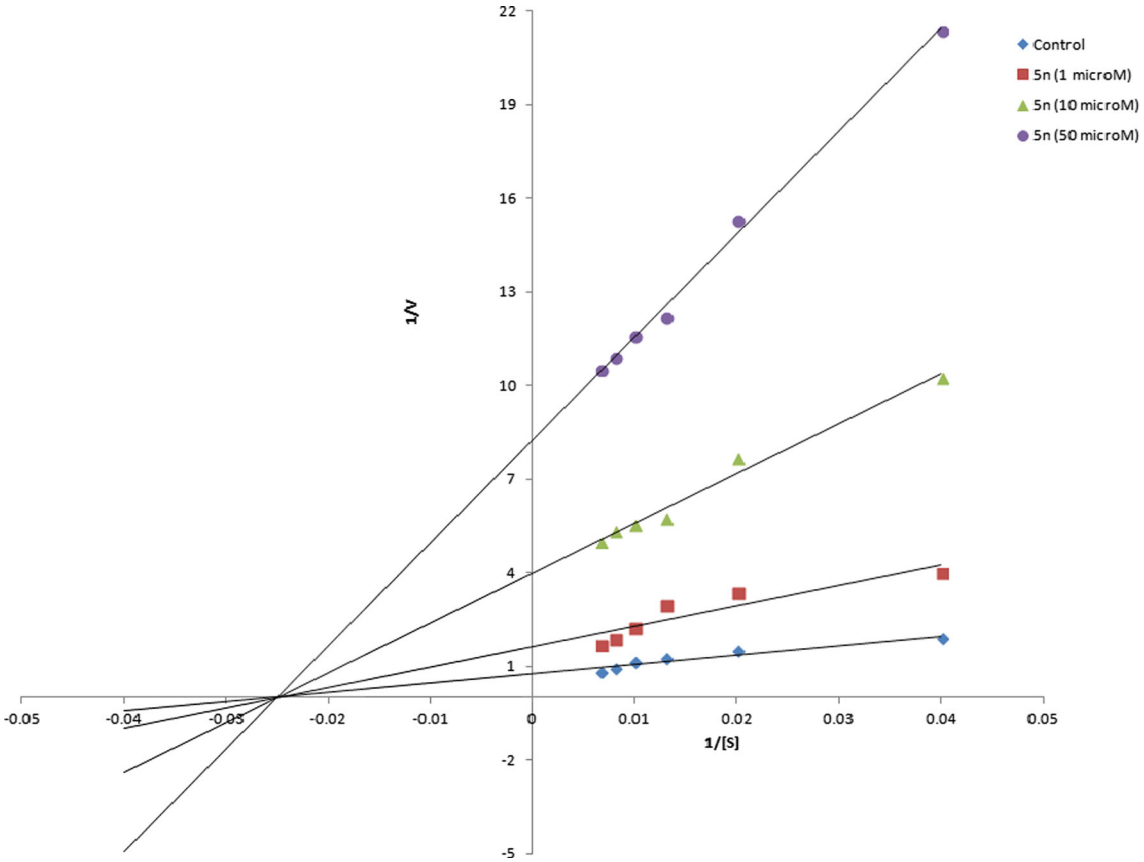


Fig. 3 Lineweaver–Burk plot

encouraged us to focus on XO inhibitors with structurally diverse and novel non-purine isosteres. Moreover, the success of febuxostat has further motivated us to focus on non-purine isosters. Febuxostat is a non-purine selective inhibitor of xanthine oxidase. It works by non-competitively blocking the molybdenum pterin centre which is the active site on xanthine oxidase. Many long- and short-term clinical trials have proved the efficacy of febuxostat in the treatment of gout and lowering uric acid levels. In these studies, febuxostat was found to be superior to allopurinol in reducing the serum uric acid levels. Thus, all these reasons have collectively led us to investigate non-competitive chemical architectures for xanthine oxidase inhibition. Keeping in view the success of some non-purine xanthine oxidase inhibitors, a library of 4*H*-pyrans was designed in the present study and the compounds were evaluated for inhibitory effects against the enzyme xanthine oxidase. All the compounds were first screened at 50 μM, and the compounds displaying a % age inhibition of >80 were further evaluated at different concentrations. Structure–activity relationship revealed that Ring A as well as Ring C remarkably influences the inhibitory potential. The most potent compound **5n** was investigated to explore the type of inhibition it was exerting, and thus, enzyme kinetics study was carried out on **5n**. The Lineweaver–Burk plot revealed that compound **5n** was a mixed-type inhibitor. The compound was studied for its interactions with the amino acid residues. The 3D structural coordinates of XO were obtained from protein databank (PDB ID: 1VDV) for the docking study. The docking study reveals that *S*-enantiomer of **5n** fits well in the binding site, while *R*-enantiomer was not able to get in the cavity (Fig. 4). *S*-enantiomer of **5n** fits well in the binding cavity and gets stabilized by various molecular interactions, i.e. "face-to-face" and "edge-to-face" pie-stacking and hydrogen bonding.

Experimental

The reagents were purchased from Sigma-Aldrich, Merck, CDH, Loba chem., Spectro chem., India, and used without further purification. All yields refer to isolated products after purification. Biotage Microwave Synthesizer (Model: Initiator) operating at 150 °C with the microwave power maximum level of 400 W. Products were characterized by spectral data. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 500 NMR Spectrometer and

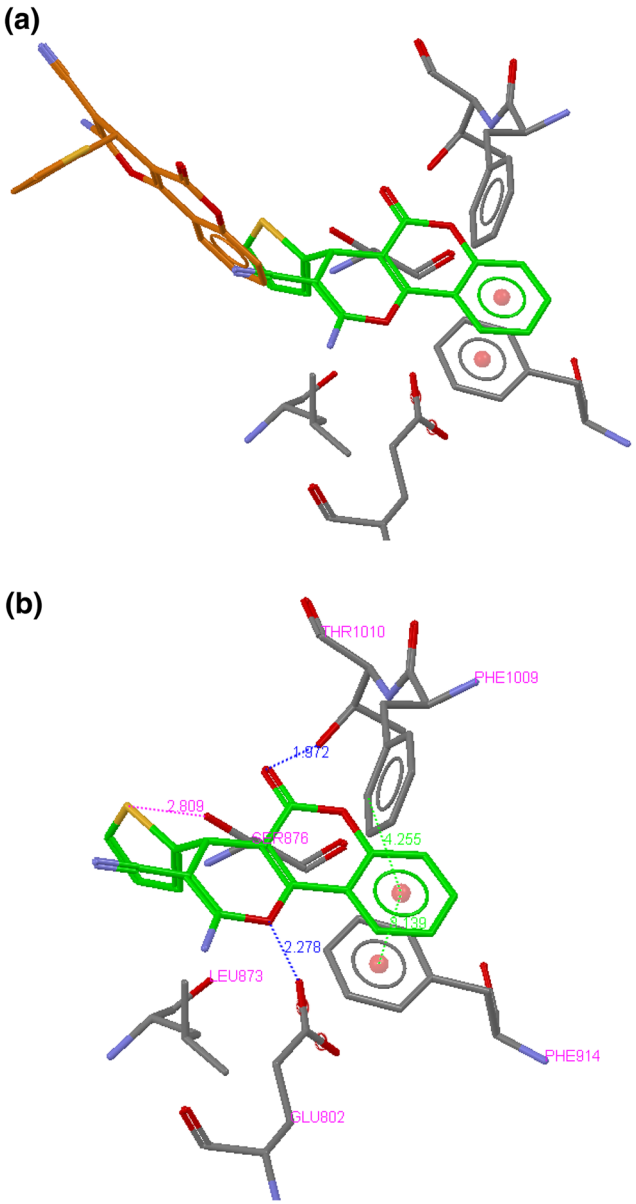


Fig. 4 **a** Binding poses of *R*-enantiomer (*brown*) and *S*-enantiomer (*green*) in binding cavity of XO. **b** Binding interactions of *S*-enantiomer of **5n** (*green*) (Color figure online)

JEOL AL 300 NMR Spectrometer. The spectra were measured in DMSO-d_6 relative to TMS (0.00 ppm). Melting points were determined in open capillaries and were uncorrected.

Experimental procedure for the synthesis of 4*H*-pyrans (1, 2, 3, 4, 5, 6, 7, 8)

A mixture of aromatic aldehyde (1 mmol), malononitrile (1 mmol), C–H-activated acidic compound (1 mmol) and catalytic amounts of DMAP (5 mol%) in a 50-ml conical flask was exposed to microwave radiation for 20 min in a microwave reactor operating at 150 °C with the maximum microwave power of 400 W. Cold methanol was added to the reaction mixture, and the solid precipitates were filtered off to obtain the desired product.

The structures of the synthesized compounds were elucidated by ^{1}H NMR and ^{13}C NMR. All spectral data were in accordance with assumed structures. In each occasion, the spectral data (^{1}H and ^{13}C NMR) of known compounds were compared with that reported in the literature.

2-Amino-5,6,7,8-tetrahydro-5-oxo-4-phenyl-4*H*-chromene-3-carbonitrile (**1a**) (Yu and Da-Ming, 2012; Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-fluorophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1b**) (Yu and Da-Ming, 2012), 2-amino-5,6,7,8-tetrahydro-4-(4-bromophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1c**) (Yu and Da-Ming, 2012), 2-amino-5,6,7,8-tetrahydro-4-(2-methoxyphenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1h**) (Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1i**) (Xu *et al*., 2011; Rostamnia and Morsali, 2014), 2-amino-5,6,7,8-tetrahydro-4-(4-nitrophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1j**) (Yu and Da-Ming, 2012; Xu *et al*., 2011; Rostamnia and Morsali, 2014; Hosseini-Monfared *et al*., 2013), 2-amino-5,6,7,8-tetrahydro-4-(4-hydroxyphenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1k**) (Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(furan-2-yl)-5-oxo-4*H*-chromene-3-carbonitrile (**1m**) (Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(3,4-dimethoxyphenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1q**) (Yu and Da-Ming, 2012; Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-chlorophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1s**) (Yu and Da-Ming, 2012; Rostamnia and Morsali, 2014; Hosseini-Monfared *et al*., 2013), 2-amino-5,6,7,8-tetrahydro-4-(3-nitrophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (**1v**) (Xu *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxo-4-phenyl-4*H*-chromene-3-carbonitrile (**2a**) (Kumar *et al*., 2009; Sadegh and Ali, 2014; Yu and Da-Ming, 2012; Gao *et al*., 2008; Hasaninejad *et al*., 2013; Jiang-Cheng *et al*., 2011; Bihani *et al*., 2013; Khaksar *et al*., 2012; Banerjee *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-fluorophenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2b**) (Sadegh and Ali, 2014; Gao *et al*., 2008; Khaksar *et al*., 2012), 2-amino-5,6,7,8-tetrahydro-4-(4-bromophenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2g**) (Sadegh and Ali, 2014; Khaksar *et al*., 2012), 2-amino-5,6,7,8-tetrahydro-4-(2-methoxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2h**) (Yu and Da-Ming, 2012; Jiang-Cheng *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2i**) (Kumar *et al*., 2009; Jiang-Cheng *et al*., 2011; Bihani *et al*., 2013; Khaksar *et al*., 2012; Banerjee *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-nitrophenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-

3-carbonitrile (**2j**) (Kumar *et al*., 2009; Sadegh and Ali, 2014; Yu and Da-Ming, 2012; Hasaninejad *et al*., 2013; Jiang-Cheng *et al*., 2011; Bihani *et al*., 2013; Khaksar *et al*., 2012; Banerjee *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-hydroxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2k**) (Gao *et al*., 2008; Jiang-Cheng *et al*., 2011; Banerjee *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(thiophen-2-yl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2n**) (Hasaninejad *et al*., 2013), 2-amino-5,6,7,8-tetrahydro-4-(3,4-methoxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2q**) (Sadegh and Ali, 2014; Jiang-Cheng *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(4-chlorophenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2s**) (Sadegh and Ali, 2014; Yu and Da-Ming, 2012; Gao *et al*., 2008; Jiang-Cheng *et al*., 2011; Bihani *et al*., 2013; Khaksar *et al*., 2012; Banerjee *et al*., 2011), 2-amino-5,6,7,8-tetrahydro-4-(3-nitrophenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (**2v**) (Kumar *et al*., 2009; Yu and Da-Ming, 2012; Jiang-Cheng *et al*., 2011; Bihani *et al*., 2013; Khaksar *et al*., 2012; Banerjee *et al*., 2011), 6-amino-2,4-dihydro-3-methyl-4-phenylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3a**) (Ali and El-Remaily, 2013; Paul *et al*., 2013; Bora *et al*., 2013; Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(4-fluorophenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3b**) (Ali and El-Remaily, 2013; Bora *et al*., 2013), 6-amino-2,4-dihydro-4-(3-hydroxyphenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3c**) (Bora *et al*., 2013), 6-amino-2,4-dihydro-4-(3-chlorophenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3d**) (Ali and El-Remaily, 2013), 6-amino-2,4-dihydro-4-(2-hydroxyphenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3f**) (Ali and El-Remaily, 2013), 6-amino-2,4-dihydro-4-(4-bromophenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3g**) (Ali and El-Remaily, 2013; Paul *et al*., 2013; Bora *et al*., 2013), 6-amino-2,4-dihydro-4-(4-methoxyphenyl)-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile (**3i**) (Ali and El-Remaily, 2013; Paul *et al*., 2013; Bora *et al*., 2013; Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(4-nitrophenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3j**) (Ali and El-Remaily, 2013; Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(4-hydroxyphenyl)-3-methylpyrano [2,3-*c*]pyrazole-5-carbonitrile (**3k**) (Ali and El-Remaily, 2013; Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(naphthalen-2-yl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3l**) (Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(thiophen-2-yl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3n**) (Paul *et al*., 2013), 6-amino-2,4-dihydro-4-(3,4-dimethoxyphenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3q**) (Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(4-chlorophenyl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3s**) (Ali and El-Remaily, 2013; Bolligarla and Das, 2011; Bihani *et al*., 2013), 6-amino-2,4-dihydro-4-(pyridin-4-yl)-3-methylpyrano[2,3-*c*]pyrazole-5-carbonitrile (**3t**) (Colombo *et al*., 2003), 2-amino-4,5-dihydro-5-oxo-4-phenylpyrano[3,2-*c*]chromene-3-carbonitrile (**5a**) (Safaei *et al*., 2012; Jain *et al*., 2013; Kidwai and Sexena, 2006), 2-amino-4,5-dihydro-4-(4-bromophenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5g**) (Safaei *et al*., 2012; Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(2-methoxyphenyl)-5-oxopyrano[3,2-c]chromene-3-carbonitrile (**5h**) (Wang *et al*., 2010), 2-amino-4,5-dihydro-4-(4-methoxyphenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5i**) (Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(4-nitrophenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5j**) (Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(4-hydroxyphenyl)-5-oxopyrano[3,2-*c*]-chromene-3-carbonitrile (**5k**) (Xiang-Shan *et al*., 2005; Kidwai and Sexena, 2006; Gong *et al*., 2009), 2-amino-4,5-dihydro-4-(furan-2-yl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5m**) (Safaei *et al*., 2012; Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(thiophen-2-yl)-5-oxopyrano[3,2-c]chromene-3-carbonitrile (**5n**) (Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(1H-indol-2-yl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5o**) (Abd-El-Aziz *et al*., 2004), 2-amino-4,5-dihydro-4-(4-hydroxy-3-methoxyphenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5p**) (Bihani *et al*., 2013), 2-amino-4,5-dihydro-4-(4-chlorophenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5s**) (Jain *et al*., 2013; Kidwai and Sexena, 2006), 2-amino-4,5-dihydro-4-(3-nitrophenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5v**) (Jain *et al*., 2013), 2-amino-4,5-dihydro-4-(2-bromophenyl)-5-oxopyrano[3,2-*c*]chromene-3-carbonitrile (**5w**) (Wang *et al*., 2010), 2-amino-4-phenyl-4*H*-benzo[*h*] chromene-3-carbonitrile (**6a**) (Bihani *et al*., 2013; Khurana *et al*., 2010), 2-amino-4-(4-fluorophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6b**) (Khurana *et al*., 2010), 2-amino-4-(4-bromophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6g**) (Khurana *et al*., 2010), 2-amino-4-(2-methoxyphenyl)-4*H*-benzo[*h*]-chromene-3-carbonitrile (**6h**) (Maalej *et al*., 2012), 2-amino-4-(4-methoxyphenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6i**) (Bihani *et al*., 2013), 2-amino-4-(4-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6j**) (Bihani *et al*., 2013; Khurana *et al*., 2010), 2-amino-4-(4-chlorophenyl)-4*H*-benzo[*h*] chromene-3-carbonitrile (**6s**) (Bihani *et al*., 2013; Khurana *et al*., 2010), 2-amino-4-(3-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (**6v**) (Bihani *et al*., 2013; Khurana *et al*., 2010), 7-amino-2,3,4,5-tetrahydro-2,4-dioxo-5-phenyl-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**7a**) (Devi *et al*., 2003), 7-amino-5-(3-chlorophenyl)-2,3,4,5-tetrahydro-2,4-dioxo-1*H*-pyrano[2,3-*d*] pyrimidine-6-carbonitrile (**7d**) (Xiang-Shan *et al*., 2005), 7-amino-5-(2-methoxyphenyl)-2,3,4,5-tetrahydro-2,4-dioxo-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**7h**) (Safaei *et al*., 2012), 7-amino-5-(4-nitrophenyl)-2,3,4,5-tetrahydro-2,4-dioxo-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**7j**) (Safaei *et al*., 2012), 7-amino-5-(4-chlorophenyl)-2,3,4,5-tetrahydro-2,4-dioxo-1*H*-pyrano[2,3-*d*]pyrimidine-6-carbonitrile (**7s**) (Safaei *et al*., 2012), 7-amino-5-(3-nitrophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-pyrano[2,3-*d*]pyrimidine-6-

carbonitrile (**7v**) (Safaei *et al*., 2012), 3-amino-1-phenyl-1*H*-benzo[*f*] chromene-2-carbonitrile (**8a**) (Bihani *et al*., 2013; Wang *et al*., 2008), 3-amino-1-(4-fluorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8b**) (Wang *et al*., 2008), 3-amino-1-(4-bromophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8g**) (Wang *et al*., 2008), 3-amino-1-(4-methoxyphenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8i**) (Wang *et al*., 2008), 3-amino-1-(4-nitrophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8j**) (Wang *et al*., 2008; Bihani *et al*., 2013), 3-amino-1-(furan-2-yl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8m**) (Wang *et al*., 2008), 3-amino-1-(4-chlorophenyl)-1*H*-benzo[*f*]-chromene-2-carbonitrile (**8s**) (Wang *et al*., 2008; Bihani *et al*., 2013), 3-amino-1-(4-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile (**8v**) (Wang *et al*., 2008; Bihani *et al*., 2013).

The characterization data for the synthesized new compounds are given below:

2-Amino-4-(3-chlorophenyl)-5,6,7,8-tetrahydro-5-oxo-4H-chromene-3-carbonitrile (**1d**) Yield 80 %; mp: 210–211 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.27–7.35 (2H, *m*), 7.12–7.18 (2H, *m*), 7.08 (2H, *bs*, D_2O exchangeable protons), 4.22 (1H, *s*), 2.63 (2H, *m*), 2.30 (2H, *t*, J = 6 Hz), 1.93 (2H, *m*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 20.22 (CH_2), 26.94 (CH_2), 35.74 (CH_2), 36.73 (CH), 57.97 (C), 113.57 (C), 120.01 (CN), 126.45 (Ar–C), 127.07 (Ar–C), 127.47 (Ar–C), 130.75 (Ar–C), 133.36 (C–Cl), 147.74 (Ar–C), 158.97 (C–C), 165.33 (C–NH_2), 196.37 (C=O). Anal. Calcd. for $C_{16}H_{13}ClN_2O_2$: C, 72.18; H, 3.94; Cl, 10.65; N, 8.42; Found: C, 72.33; H, 3.58; Cl, 10.95; N, 8.56.

2-Amino-4-(3,4-dihydroxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**1e**) Yield 60 %; mp: 200–201 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 6.86 (2H, *s*, D_2O exchangeable protons), 6.61 (1H, *d*, J = 8.1 Hz,), 6.54 (1H, *s*), 6.41 (1H, *d*, J = 8.1 Hz), 4.01 (1H, *s*), 2.58 (2H, *bs*), 2.26 (2H, *bs*), 1.92 (2H, *m*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 19.39 (CH_2), 26.17 (CH_2), 36.85 (CH_2), 38.63 (CH), 58.19 (C), 113.96 (C), 120.27 (CN), 118.18 (Ar–C), 116.2 (Ar–C), 123.14 (Ar–C), 136.22 (Ar–C), 144.21 (C–OH), 147.27 (C–OH), 158.12 (C), 165.33 (C–NH_2), 196.97 (C=O). Anal. Calcd. for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39; Found: C, 64.24; H, 5.11; N, 9.55.

2-Amino-4-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**1l**) Yield 83 %; mp: 210–211 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.39 (1H, *d*, J = 8.1 Hz), 7.90 (1H, *d*, J = 1.5 Hz), 7.77 (1H, *d*, J = 8.1 Hz), 7.57 (1H, *d*, J = 5.1 Hz), 7.41–7.54 (3H, *m*), 7.25 (1H, *d*, J = 6.9 Hz), 6.95 (2H, *s*, D_2O exchangeable protons), 5.15 (1H, *s*), 2.71 (2H, *m*), 2.30 (2H, *m*), 1.98 (2H, *m*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 20.31 (CH_2), 26.74 (CH_2), 35.69 (CH_2), 36.78 (CH), 58.88 (C), 113.69 (C), 117.33(CN), 124.52 (Ar–C), 124.96 (Ar–C), 125.43 (Ar–C), 125.67 (Ar–C), 126.73 (Ar–C), 126.86 (Ar–C), 128.94 (Ar–C), 132.66 (Ar–C), 133.57 (Ar–C), 134.01 (Ar–C), 158.67 (C), 165.98 (C–NH_2), 196.77 (C=O). Anal. Calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86; Found: C, 75.20; H, 5.30; N, 9.10.

2-Amino-4-(thiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**1n**) Yield 85 %; mp: 158–159 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.38 (1H, *d*, J = 4.8 Hz), 7.119 (2H, *s*, D_2O exchangeable protons), 6.85–6.92 (2H, *m*), 4.53 (1H, *s*), 2.56 (2H, *bs*), 2.31 (2H, *bs*), 1.90 (2H, *m*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 20.33 (CH_2), 25.64 (CH), 26.96 (CH_2), 36.87 (CH_2), 58.44 (C), 113.74 (C), 120.22 (CN), 123.67 (Ar–C), 126.61 (Ar–C), 126.98 (Ar–C), 139.77 (Ar–C), 158.71 (C), 165.83 (C–NH_2), 196.27 (C=O). Anal. Calcd. for $C_{14}H_{12}N_2O_2S$: C, 61.75; H, 4.44; N, 10.29; S, 11.77; Found: C, 61.90; H, 4.35; N, 10.45; S, 11.81.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**1p**) Yield 76 %; mp: 200–201 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.87 (1H, *s*, D_2O exchangeable proton), 6.91 (2H, *bs*, D_2O exchangeable proton), 6.67 (2H, *m*), 6.51 (1H, *d*, J = 8.1 Hz), 4.09 (1H, *s*), 3.72 (3H, *s*), 2.59 (2H, *bs*), 2.27 (2H, *bs*), 1.91 (2H, *m*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 20.42 (CH_2), 26.80(CH_2), 36.24(CH_2), 37.37 (CH), 56.22 (OCH_3), 58.78 (C), 113.11 (C), 114.22 (Ar–C), 116.17 (Ar–C), 120.99 (CN), 122.88 (Ar–C), 135.42 (Ar–C), 142.23 (C–OH), 151.39 (C–OCH_3), 158.91 (C), 166.01 (C–NH_2), 196.72 (C=O). Anal. Calcd. for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97; Found: C, 64.80; H, 5.02; N, 8.93.

2-Amino-4-(3-methylthiophen-2-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**1u**) Yield 78 %; mp: 150–151 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.19 (1H, *d*, J = 4.8 Hz), 7.01 (2H, *s*, D_2O exchangeable protons), 6.74 (1H, *d*, J = 5.1 Hz), 4.57 (1H, *s*), 2.60 (2H, *bs*), 2.28 (2H, *bs*), 2.23 (3H, *s*), 1.96 (2H, *bs*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 12.33 (CH_3), 19.10 (CH_2), 20.30 (CH), 26.44 (CH_2), 36.22 (CH_2), 58.65 (C), 113.99 (C), 120.27 (CN), 124.10 (Ar–C), 124.70 (Ar–C), 135.11 (Ar–C), 135.99 (Ar–C), 158.31 (C), 166.21 (C–NH_2), 196.11 (C=O). Anal. Calcd. for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78; S, 11.20; Found: C, 63.21; H, 4.58; N, 9.94; S, 11.32.

2-Amino-4-(3,4-dihydroxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**2e**) Yield 57 %; mp: 185–186 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.78 (2H, *bs*, D_2O exchangeable protons), 6.89 (2H, *s*, D_2O exchangeable protons), 6.61 (1H, *d*, J = 8.1 Hz), 6.52 (1H, *d*, J = 1.8 Hz), 6.38 (1H, *dd*, J = 1.8 and 8.1 Hz), 3.97 (1H, *s*), 2.55 (2H, *bs*), 2.22 (1H, *d*,

$J = 16.2$ Hz), 2.08 (1H, *d*, $J = 16.2$ Hz), 1.06 (3H, s), 0.92 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 27.23 (CH_3), 28.95 (CH_3), 32.25 (C), 35.27 (CH_2), 50.52 (CH_2), 59.30 (C), 113.79 (C), 115.12 (Ar–C), 115.74 (Ar–C), 118.36 (CN), 123.1 (Ar–C), 136.27 (Ar–C), 144.40 (C–OH), 145.39 (C–OH), 158.87 (C), 162.31 (C–NH_2), 196.11 (C=O). Anal. Calcd. for $C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58; Found: C, 65.98; H, 5.75; N, 8.32.

2-Amino-4-(2-hydroxyphenyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**2f**) Yield 68 %; mp: 80–81 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.72 (1H, *s*, D_2O exchangeable proton), 6.32–6.89 (7H, *m*), 4.84 (1H, *s*), 2.55 (2H, *bs*), 2.22 (1H, *d*, $J = 16.2$ Hz), 2.08 (1H, *d*, $J = 16.2$ Hz), 1.06 (3H, *s*), 0.92 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 27.23 (CH_3), 29.05 (CH_3), 32.46 (C), 35.78 (C), 50.15 (CH_2), 59.36 (C), 113.23 (C), 115.25 (Ar–C), 118.63 (CN), 121.22 (Ar–C), 122.36 (Ar–C), 127.78 (Ar–C), 130.20 (Ar–C), 147.41 (C), 158.68 (C–OH), 162.26 (C–NH_2), 196.01 (C=O). Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 72.95; H, 6.80; N, 9.45; Found: C, 72.17; H, 7.10; N, 9.64.

6-Amino-4-(2-methoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**3h**) Yield 84 %; mp: 170–171 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 12.01 (1H, *s*, D_2O exchangeable proton), 7.19 (1H, *m*), 6.96–7.01 (2H, *m*), 6.90 (1H, *m*), 6.79 (2H, *s*, D_2O exchangeable protons), 4.97 (1H, *s*), 3.78 (3H, *s*), 1.79 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 10.55 (CH_3), 11.10 (CH), 54.45 (OCH_3), 56.11 (C), 112.75 (Ar–C), 110.96 (Ar–C), 114.90 (C), 120.99 (Ar–C), 121.00 (CN), 126.80 (Ar–C), 130.01 (Ar–C), 136.82 (C), 142.27 (C–OCH_3), 156.09 (C), 162.00 (C–NH_2). Anal. Calcd. for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85; Found: C, 64.09; H, 4.75; N, 19.76.

6-Amino-4-(furan-2-yl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**3m**) Yield 67 %; mp: 185–186 (DEC) °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 12.16 (1H, *s*, D_2O exchangeable proton), 7.53 (1H, *bs*), 6.95 (2H, *s*, D_2O exchangeable proton), 6.37 (1H, *d*, $J = 1.8$ Hz), 6.17 (1H, *d*, $J = 2.7$ Hz), 4.77 (1H, *s*), 1.97 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 10.03 (CH_3), 30.28 (CH), 54.45 (C), 95.57 (C), 106.09 (Ar–C), 110.69 (Ar–C), 121.04 (CN), 136.28 (C), 142.72 (Ar–C), 155.27 (Ar–C), 156.18 (C), 161.94 (C–NH_2). Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13; Found: C, 59.81; H, 3.97; N, 23.29.

6-Amino-4-(1H-indol-2-yl)-3-methyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**3o**) Yield 66 %; mp: 190–191 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.69 (1H, *s*, D_2O exchangeable proton), 8.91 (1H, *s*, D_2O exchangeable proton), 8.34 (1H, *d*, $J = 7.2$ Hz), 7.92 (1H, *s*), 7.48 (1H, *d*, $J = 7.5$ Hz), 7.21–7.29 (2H, *m*), 4.84 (1H, *s*), 1.76 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 10.88 (CH_3), 30.02 (CH), 54.44 (C), 96.03 (C), 105.55 (Ar–C), 110.66 (Ar–C), 119.94 (Ar–C), 120.15 (CN), 120.88 (Ar–C), 122.22 (Ar–C), 128.04 (Ar–C), 135.20 (Ar–C), 136.55 (Ar–C), 142.05 (C), 162.27 (C–NH_2). Anal. Calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04; Found: C, 65.66; H, 4.33; N, 24.13.

6-Amino-3-methyl-4-(2,3,4-trimethoxyphenyl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**3r**) Yield 79 %; mp: 196–197 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 12.10 (1H, *s*, D_2O exchangeable protons), 6.87 (2H, D_2O exchangeable protons), 6.43 (2H, *bs*), 4.58 (1H, *s*), 3.85 (6H, *s*), 3.84 (3H, *s*), 1.87 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 10.53 (CH_3), 12.01 (CH), 54.28 (OCH_3), 55.63 (C), 56.27 (OCH_3), 56.36 (OCH_3), 96.75 (C), 105.30 (Ar–C), 110.96 (Ar–C), 120.69 (CN), 123.36 (Ar–C), 136.42 (C), 139.91 (C–OCH_3), 142.94 (C–OCH_3), 147.71 (C–OCH_3), 151.72 (C), 162.76 (C–NH_2). Anal. Calcd. for $C_{17}H_{18}N_4O_4$: C, 59.64; H, 5.30; N, 16.37; Found: C, 59.78; H, 5.25; N, 16.55.

6-Amino-3-methyl-4-(3-methylthiophen-2-yl)-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**3u**) Yield 85 %; mp: 179–180 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 12.15 (1H, *s*, D_2O exchangeable proton), 7.02(1H, *d*, $J = 5.1$ Hz), 6.86 (2H, *bs*, D_2O exchangeable protons), 6.78 (1H, *d*, $J = 5.1$ Hz), 5.01 (1H, *s*), 2.16 (3H, *s*), 1.83 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 10.35 (CH_3), 11.55 (CH_3), 12.70 (CH), 54.90 (C), 110.76 (C), 120.07 (CN), 124.11 (Ar–C), 124.71 (Ar–C), 133.31 (Ar–C), 135.56 (Ar–C), 136.89 (C), 142.24 (C), 162.08 (C–NH_2). Anal. Calcd. for $C_{13}H_{12}N_4OS$: C, 57.34; H, 4.44; N, 20.57; S, 11.77; Found: C, 56.99; H, 4.71; N, 20.34; S, 11.98.

7-Amino-2-oxo-5-phenyl-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4a**) Yield 82 %; mp: 180–181 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.98 (2H, *d*, $J = 7.5$ Hz), 7.19 (2H, *d*, $J = 7.5$ Hz), 4.56 (1H, *s*), 3.89 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, v TMS = 0): 36.53 (CH), 59.63 (C), 94.62 (C), 115.17 (CN), 125.88 (Ar–C), 126.71 (Ar–C), 126.77 (Ar–C), 142.25 (Ar–C), 151.99 (C=O), 158.36 (C), 160.36 (C–NH_2), 174.62 (C=S). Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C, 56.37; H, 3.38; N, 18.78; S, 10.75; Found: C, 56.60; H, 3.15; N, 18.92; S, 10.82.

7-Amino-5-(3-chlorophenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4d**) Yield 87 %; mp: 190–191 °C, ^{1}H NMR (DMSO-d_6, 300 MHz): δ, TMS = 0): 9.11 (1H, *s*), 10.91 (1H, *s*), 7.41 (1H, *s*), 7.24–7.33 (2H, *m*), 7.13 (1H, *d*, $J = 8.3$ Hz), 7.06 (1H, *s*), 4.59 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ,

TMS = 0): 36.86 (CH), 59.97 (C), 94.75 (C), 115.67 (CN), 125.61 (Ar–C), 127.63 (Ar–C), 128.94 (Ar–C), 130.26 (Ar–C), 134.43 (C–Cl), 143.34 (Ar–C), 151.69 (C=O), 158.24 (C), 160.56 (C–NH_2), 174.67 (C=S). Anal. Calcd. for $C_{14}H_9ClN_4O_2S$: C, 50.53; H, 2.73; Cl, 10.65; N, 16.84; S, 9.64; Found: C, 50.80; H, 2.51; Cl, 10.76; N, 16.96; S, 9.38.

7-Amino-5-(4-bromophenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4g**) Yield 88 %; mp: 185–186 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.17 (1H, *s*, D_2O exchangeable proton), 7.47 (2H, *d*, *J* = 8.1 Hz), 7.18 (2H, *d*, *J* = 8.4 Hz), 7.15 (2H, *bs*, D_2O exchangeable proton), 4.23 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.86 (CH), 59.97 (C), 94.75 (C), 115.67 (CN), 120.55 (C–Br), 131.25 (Ar–C), 131.32 (Ar–C), 131.51 (Ar–C), 131.82 (Ar–C), 141.24 (Ar–C), 151.37 (C=O), 158.59 (C), 160.86 (C–NH_2), 174.78 (C=S). Anal. Calcd. for $C_{14}H_9BrN_4O_2S$: C, 44.58; H, 2.40; Br, 21.18; N, 14.85; S, 8.50; Found: C, 44.81; H, 2.12; Br, 21.36; N, 14.52; S, 8.39.

7-Amino-5-(2-methoxyphenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4h**) Yield 80 %; mp: 180–181 °C, 1H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 10.88 (1H, *s*), 9.01 (1H, *s*), 7.13 (1H, *d*, *J* = 8.0 Hz), 6.87–6.89 (3H, *m*), 6.84–6.88 (2H, *m*), 4.29 (1H, *s*), 3.64 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.42 (CH), 56.31 (OCH_3), 59.78 (C), 94.79 (C), 114.41 (Ar–C), 115.99 (CN), 121.58 (Ar–C), 121.89 (Ar–C), 126.88 (Ar–C), 130.12 (Ar–C), 151.98 (C=O), 157.66 (C), 158.37 (C–OCH_3), 160.00 (C–NH_2), 174.28 (C=S). Anal. Calcd. for $C_{15}H_{12}N_4O_3S$: C, 54.87; H, 3.68; N, 17.06; S, 9.77; Found: C, 54.44; H, 3.92; N, 17.32; S, 9.49.

7-Amino-5-(4-methoxyphenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4i**) Yield 76 %; mp: 200–201 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.98 (2H, *d*, *J* = 7.5 Hz), 7.19 (2H, *d*, *J* = 7.5 Hz), 4.56 (1H, *s*), 3.89 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.35 (CH), 56.42 (OCH_3), 59.36 (C), 94.26 (C), 113.96 (Ar–C), 114.13 (Ar–C), 115.71 (CN), 129.00 (Ar–C), 129.54 (Ar–C), 136.08 (Ar–C), 151.90 (C=O), 157.76 (C), 158.63 (C–OCH_3), 160.63 (C–NH_2), 174.26 (C=S). Anal. Calcd. for $C_{15}H_{12}N_4O_3S$: C, 54.87; H, 3.68; N, 17.06; S, 9.77; Found: C, 54.51; H, 3.88; N, 17.34; S, 9.82.

7-Amino-5-(4-nitrophenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4j**) Yield 84 %; mp: 268–269 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 10.93 (1H, *s*), 9.41 (1H, *s*), 8.13 (2H, *d*, *J* = 8.5 Hz), 7.49 (2H, *d*, *J* = 8.5 Hz), 7.08 (2H, *s*), 3.29 (1H, *s*). ^{13}C NMR (DMSO, 500 MHz, δ, TMS = 0): 36.59 (CH), 59.23 (C), 94.28 (C), 115.58 (CN), 121.57 (Ar–C), 121.89 (Ar–C), 130.28 (Ar–C), 130.85 (Ar–C), 145.14 (Ar–C), 148.95 (C=O), 151.39 (Ar–C), 158.27 (C), 160.33 (C–NH_2), 174.68 (C=S). Anal. Calcd. for $C_{14}H_9N_5O_4S$: C, 48.98; H, 2.64; N, 20.40; S, 9.34; Found: C, 49.12; 2.36; N, 20.54; S, 9.60.

7-Amino-2-oxo-5-(thiophen-2-yl)-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4n**) Yield 81 %; mp: 212–213 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 12.09 (1H, *bs*), 11.42 (1H, *bs*), 7.38 (2H, *bs*), 7.02 (1H, *d*), 6.57 (1H, *m*), 6.49 (1H, *d*), 4.21 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 34.33 (CH), 59.65 (C), 94.56 (C), 115.69 (CN), 123.65 (Ar–C), 126.71 (Ar–C), 126.99 (Ar–C), 139.77 (Ar–C), 151.91 (C=O), 158.79 (C), 160.45 (C–NH_2), 174.85 (C=S). Anal. Calcd. for $C_{12}H_8N_4O_2S_2$: C, 47.36; H, 2.65; N, 18.41; S, 21.07; Found: C, 47.56; H, 2.45; N, 18.19; S, 21.29.

7-Amino-5-(4-chlorophenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4s**) Yield 85 %; mp: 194–195 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.1 (1H, *s*), 9.2 (1H, *s*), 7.73 (2H, *d*, *J* = 8.0 Hz), 7.17 (2H, *d*, *J* = 8.0 Hz), 6.97 (2H, *s*), 4.37 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.97 (CH), 59.60 (C), 94.50 (C), 115.11 (CN), 128.48 (Ar–C), 128.84 (Ar–C), 130.01 (Ar–C), 130.10 (Ar–C), 131.53 (C–Cl), 140.30 (Ar–C), 151.97 (C=O), 158.15 (C), 160.17 (C–NH_2), 174.19 (C=S). Anal. Calcd. for $C_{14}H_9ClN_4O_2S$: C, 50.53; H, 2.73; Cl, 10.65; N, 16.84; S, 9.64; Found: C, 50.81; H, 2.39; Cl, 10.54; N, 16.78; S, 9.78.

7-Amino-5-(3-methylthiophen-2-yl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4u**) Yield 80 %; mp: 228–229 °C, ^{1}H NMR (DMSO-d_6, 300, δ, TMS = 0): 12.04 (1H, *bs*), 11.02 (1H, *bs*), 7.26 (2H, *bs*), 6.48 (1H, *d*), 6.62 (1H, *m*), 3.99 (1H, *s*), 2.20 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 13.13 (CH_3), 33.03 (CH), 59.32 (C), 94.43 (C), 115.54 (CN), 124.11 (Ar–C), 124.17 (Ar–C), 133.95 (Ar–C), 135.09 (Ar–C), 151.15 (C=O), 158.59 (C), 160.19 (C–NH_2), 174.91 (C=S). Anal. Calcd. for $C_{13}H_{10}N_4O_2S_2$: C, 51.65; H, 3.33; N, 18.53; S, 10.61; Found: C, 51.39; H, 3.61; N, 18.72; S, 10.31.

7-Amino-5-(3-nitrophenyl)-2-oxo-4-thioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**4v**) Yield 80 %; mp: 200–201 °C, ^{1}H NMR (DMSO-d_6, 300, δ, TMS = 0): 11.13 (1H, *bs*), 9.15 (1H, *s*), 8.16 (1H, *s*), 8.01 (1H, *d*, *J* = 8.0 Hz), 7.56–7.63 (2H, *m*), 4.33 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.99 (CH), 59.95 (C), 94.55 (C), 115.16 (CN), 118.28 (Ar–C), 124.34 (Ar–C), 129.29 (Ar–C), 135.45 (Ar–C), 143.53 (Ar–C), 150.60 (C–NO_2), 151.06 (C=O), 158.84 (C), 160.48 (C–NH_2), 174.88 (C=S). Calculated Anal. Calcd. for

$C_{14}H_9N_5O_4S$: C, 48.98; H, 2.64; N, 20.40; S, 9.34; Found: C, 49.10; H, 2.34; N, 20.68; S, 9.02.

2-Amino-4,5 dihydro-4-(4-fluorophenyl)-5-oxopyrano[3,2-c]chromene-3-carbonitrile (**5b**) Yield 84 %; mp: 190–191 °C, 1H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.83 (1H, *d*, $J = 8.1$ Hz), 7.65 (1H, *dd*, $J = 8.1$ and 7.1 Hz), 7.44 (1H, *m*), 7.32 (1H, *d*, $J = 8.4$ Hz), 7.21 (2H, *d*, $J = 7.9$ Hz), 7.12 (2H, *d*, $J = 8.0$ Hz), 4.31 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 37.81 (CH), 58.14 (C), 105.24 (C), 115.21 (Ar–C), 115.41 (Ar–C), 115.45 (Ar–C), 116.40 (Ar–C), 119.14 (CN), 123.32 (Ar–C), 125.40 (Ar–C), 128.34 (Ar–C), 130.58 (Ar–C), 130.60 (Ar–C), 139.12 (Ar–C), 152.50 (Ar–C), 159.10 (C–NH2), 159.90 (C–F), 160.12 (C), 161.90 (C=O). Anal. Calcd. for $C_{19}H_{11}FN_2O_3$: C, 68.26; H, 3.32; F, 5.68; N, 8.38; Found: C, 68.32; H, 3.28; F, 5.70; N, 8.44.

2-Amino-4-(naphthalene-2-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**5l**) Yield 88 %; mp: 210–211 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.43 (1H, *d*, $J = 7.8$ Hz), 7.94–7.99 (2H, *m*), 7.82 (1H, *d*, $J = 8.1$ Hz), 7.72 (1H, *m*), 7.44–7.62 (5H, *m*),7.32–7.41 (3H, *m*), 5.47 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 36.87 (CH), 58.16 (C), 104.03 (C), 117.30 (Ar–C), 119.66 (CN), 121.55 (Ar–C), 125.66 (Ar–C), 125.79 (Ar–C), 126.15 (Ar–C), 126.71 (Ar–C), 127.32 (Ar–C), 127.43 (Ar–C), 127.76 (Ar–C), 127.89 (Ar–C), 131.65 (Ar–C), 133.94 (Ar–C), 135.35 (Ar–C), 152.56 (Ar–C), 153.49 (C–NH_2), 158.98 (Ar–C), 160.10 (C=O). Anal. Calcd. for $C_{23}H_{14}N_2O_3$: C, 75.40; H, 3.85; N, 7.65; Found: C, 75.26; H, 4.00; N, 7.35.

2-Amino-4-(3,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**5q**) Yield 76 %; mp: 170–171 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.92 (1H, *d*, $J = 8.1$ Hz), 7.66 (1H, *dd*, $J = 8.1$ Hz and 7.2 Hz), 7.44 (1H, *m*), 7.37 (1H, *d*, $J = 9$ Hz), 6.83–6.86 (2H, *m*), 6.76 (1H, *d*, $J = 8.7$ Hz), 4.43 (1H, *s*), 3.73 (3H, *s*), 3.76 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 36.98 (CH), 55.97 (OCH_3), 56.02 (OCH_3), 58.61 (C), 104.59 (C), 112.11 (Ar–C), 112.36 (Ar–C), 113.51 (Ar–C), 117.03 (Ar–C), 119.76 (CN), 120.14 (Ar–C), 122.93 (Ar–C), 125.10 (Ar–C), 133.31 (Ar–C), 136.31 (Ar–C), 148.42 (Ar–C), 148.99 (Ar–C), 152.58 (C), 153.64 (C–NH_2), 158.39 (C), 160.00 (C=O). Anal. Calcd. for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44; Found: C, 67.26; H, 3.98; N, 7.64.

2-Amino-4-(3-chlorophenyl)-4H-benzo[h]chromene-3-carbonitrile (**6d**) Yield 82 %; mp: 176–177 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.25 (1H, *d*, $J = 8.1$ Hz), 7.89 (1H, *d*, $J = 7.8$ Hz), 7.56–7.67 (3H, *m*), 7.12 (1H, *d*, $J = 8.4$ Hz), 4.97 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 29.33 (CH), 56.10 (C), 117.67 (CN), 121.20 (Ar–C), 124.56 (Ar–C), 126.49 (Ar–C), 126.97 (Ar–C), 127.23 (Ar–C), 127.47 (Ar–C), 127.86 (Ar–C), 128.18 (Ar–C), 131.20 (Ar–C), 133.25 (Ar–C), 133.73 (C–Cl), 143.25 (Ar–C), 148.61 (Ar–C), 160.74 (C–NH_2). Anal. Calcd. for $C_{20}H_{13}ClN_2O$: C, 72.18; H, 3.94; Cl, 10.65; N, 8.42; Found: C, 71.94; H, 3.63; Cl, 10.86; 8.22.

2-Amino-4-(3,4-dihydroxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (**6e**) Yield 71 %; mp: 150–151 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.30–8.39 (2H, *m*), 7.83–7.97 (2H, *m*), 7.18–7.73 (4H, *m*), 6.92 (1H, *d*, $J = 8.4$ Hz), 7.18 (2H, *s*, D_2O exchangeable protons), 5.80 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 30.10 (CH), 59.22 (C), 115.36 (Ar–C), 117.76 (CN), 117.96 (Ar–C), 120.23 (Ar–C), 120.93 (Ar–C), 121.10 (Ar–C), 122.35 (Ar–C), 125.71 (Ar–C), 125.74 (Ar–C), 127.48 (Ar–C), 132.77 (Ar–C), 134.44 (Ar–C), 143.05 (Ar–C), 144.20 (C–OH), 147.55 (C–OH), 160.31 (C–NH_2). Anal. Calcd. for $C_{20}H_{14}N_2O_3$: C, 72.72; H, 4.27; N, 8.48; Found: C, 72.99; H, 3.98; N, 8.72.

2-Amino-4-(4-hydroxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (**6k**) Yield 85 %; mp: 228–229 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 9.35 (1H, *s*, D_2O exchangeable protons), 8.23 (1H, *d*, $J = 8.1$ Hz), 7.87 (1H, *d*, $J = 8.1$ Hz), 7.57–7.63 (3H, *m*), 7.03–7.11 (5H, *m*), 6.69(2H, *d*, $J = 8.1$ Hz), 4.77 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 29.88 (CH), 57.24 (C), 115.83 (Ar–C), 118.92 (CN), 121.14 (Ar–C), 123.23 (Ar–C), 124.84 (Ar–C), 126.80 (Ar–C), 127.07 (Ar–C), 129.14 (Ar–C), 133.06 (Ar–C), 136.64 (Ar–C), 143.02 (Ar–C), 151.74 (C–OH), 160.43 (C–NH_2). Anal. Calcd. for $C_{20}H_{14}N_2O_2$: C, 76.42; H, 4.49; N, 8.91; Found: C, 76.22; H, 4.64; N, 9.02.

2-Amino-4-(thiophen-2-yl)-4H-benzo[h]chromene-3-carbonitrile (**6n**) Yield 83 %; mp: 231–232 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.22 (1H, *d*, $J = 8.1$ Hz), 7.89 (1H, *d*, $J = 7.8$ Hz), 7.55–7.65 (3H, *m*), 7.36 (1H, *d*, $J = 4.5$ Hz), 7.24–7.28 (3H, *m*), 7.08 (1H, *bs*), 6.93 (1H, *bs*), 5.26 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 30.53 (CH), 59.02 (C), 119.84 (CN), 120.44 (Ar–C), 120.97 (Ar–C), 121.12 (Ar–C), 123.67 (Ar–C), 125.84 (Ar–C), 125.98 (Ar–C), 126.37 (Ar–C), 126.63 (Ar–C), 126.74 (Ar–C), 132.77 (Ar–C), 139.44 (Ar–C), 143.50 (Ar–C), 160.77 (C–NH_2). Anal. Calcd. for $C_{18}H_{12}N_2OS$: C, 71.03; H, 3.97; N, 9.20; S, 10.54; Found: C, 71.38; H, 3.58; N, 8.90; S, 10.82.

2-Amino-4-(3,4-dimethoxyphenyl)-4H-benzo[h]chromene-3-carbonitrile (**6q**) Yield 73 %; mp: 140–141 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.24 (1H, *d*, $J = 7.8$ Hz), 7.88(1H, *d*, $J = 7.54$ Hz), 7.54–7.65 (3H, *m*), 7.11–7.16 (3H, *m*), 6.72–6.91 (3H, *m*), 4.84 (1H, *s*), 3.82

(3H, *s*), 3.80 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 31.11 (CH), 55.67 (OCH_3), 56.66 (OCH_3), 59.68 (C), 113.32 (Ar–C), 115.86 (Ar–C), 118.59 (Ar–C), 120.24 (Ar–C), 120.95 (Ar–C), 121.17 (Ar–C), 125.71 (Ar–C), 125.77 (Ar–C), 127.42 (Ar–C), 132.76 (Ar–C), 133.75 (Ar–C), 143.54 (Ar–C), 147.84 (C–OCH_3), 150.31 (C–OCH_3), 160.34 (C–NH_2). Anal. Calcd. for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82; Found: C, 73.44; H, 5.26; N, 8.10.

7-Amino-5-(4-bromophenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**7g**) Yield 79 %; mp: 170–171 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.17 (1H, *s*, D_2O exchangeable proton), 7.47 (2H, *d*, $J = 8.1$ Hz), 7.18 (2H, *d*, $J = 8.4$ Hz), 7.15 (2H, *bs*, D_2O exchangeable proton), 4.23 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.72 (CH), 58.72 (C), 88.40 (C), 119.51 (CN), 120.22 (C–Br), 130.15 (Ar–C), 131.58 (Ar–C), 144.06 (Ar–C), 149.95 (C=O), 152.81 (C–NH_2), 158.00 (C), 162.96 (C=O). Anal. Calcd. for $C_{14}H_9BrN_4O_3$: C, 46.56; H, 2.51; Br, 22.12; N, 15.51; Found: C, 46.26; H, 2.86; Br, 22.31; N, 15.23.

7-Amino-5-(4-methoxyphenyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**7i**) Yield 70 %; mp: 237–238 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.98 (2H, *d*, $J = 7.5$ Hz), 7.19 (2H, *d*, $J = 7.5$ Hz), 4.56 (1H, *s*), 3.89 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 35.27 (CH), 55.55 (OCH_3), 58.22 (C), 88.44 (C), 114.22 (Ar–C), 120.15 (CN), 130.11 (Ar–C), 134.50 (Ar–C), 149.59 (C=O), 152.18 (C–OCH_3), 156.00 (C–NH_2), 158.55 (C), 162.69 (C=O). Anal. Calcd. for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94; Found: C, 57.99; H, 3.96; N, 17.52.

7-Amino-5-(thiophen-2-yl)-2,3,4,5-tetrahydro-2,4-dioxo-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**7n**) Yield 74 %; mp: 172–173 °C, 1H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.14 (1H, *s*, D_2O exchangeable proton), 7.40 (1H, *d*, $J = 7.8$ Hz), 6.83–6.86 (2H, *bs*), 6.8 (1H, *s*, D_2O exchangeable proton), 4.52 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 25.80 (CH), 58.10 (C), 80.10 (C), 119.10 (CN), 123.50 (Ar–C), 125.40 (Ar–C), 127.00 (Ar–C), 139.80 (Ar–C), 150.40 (C=O), 158.90 (C–NH_2), 160.84 (C), 163.68 (C=O). Anal. Calcd. for $C_{12}H_8N_4O_3S$: C, 50.00; H, 2.80; N, 19.43; S, 11.12; Found: C, 48.10; H, 3.21; N, 20.21; S, 11.21.

7-Amino-5-(3-methylthiophen-2-yl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-pyrano[2,3-d]pyrimidine-6-carbonitrile (**7u**) Yield 78 %; mp: 171–173 °C, 1H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 11.21 (1H, *s*, D_2O exchangeable proton), 7.80 (2H, *bs*, D_2O exchangeable proton), 7.28 (1H, *d*, $J = 7.6$ Hz), 6.26 (1H, *d*, 7.6 Hz), 4.62 (1H, *s*), 2.30 (3H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 13.40 (CH_3), 22.30 (CH), 58.10 (C), 80.12 (C), 119.10 (CN), 121.10 (Ar–C), 124.30 (Ar–C), 133.60 (Ar–C), 135.40 (Ar–C), 150.6 (C=O), 159.3 (C–NH_2), 160.4 (C), 163.8 (C=O). Anal. Calcd. for $C_{13}H_{10}N_4O_3S$: C, 51.65; H, 3.33; N, 18.53; S, 10.61; Found: C, 50.21; H, 3.20; N, 20.10; S, 12.31.

3-Amino-1-(2-methoxyphenyl)-1H-benzo[f]chromene-2-carbonitrile (**8h**) Yield 70 %; mp: 200–201 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 7.89–7.92 (2H, *m*), 7.74 (1H, *d*, $J = 7.8$ Hz), 7.42 (2H, *m*), 7.32 (1H, *dd*, $J = 1.2$ and 6.9 Hz), 7.13 (1H, *m*), 7.03 (1H, *d*, $J = 8.4$ Hz), 6.77–6.86 (4H, *m*), 5.60 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 17.31 (CH), 56.44 (OCH_3), 57.41 (C), 112.18 (Ar–C), 117.16 (CN), 121.52 (Ar–C), 123.39 (Ar–C), 125.30 (Ar–C), 127.61 (Ar–C), 128.41 (Ar–C), 128.95 (Ar–C), 129.05 (Ar–C), 129.63 (Ar–C), 131.13 (Ar–C), 134.07 (Ar–C), 151.77 (Ar–C), 156.11 (C–OCH_3), 160.69 (C–NH_2). Anal. Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53; Found: C, 76.52; H, 5.12; N, 8.88.

3-Amino-1-(thiophen-2-yl)-1H-benzo[f]chromene-2-carbonitrile (**8n**) Yield 77 %; mp: 228–229 °C, ^{1}H NMR (DMSO-d_6, 300 MHz, δ, TMS = 0): 8.05 (1H, *d*, $J = 8.1$ Hz), 7.93 (2H, *d*, $J = 8.7$ Hz), 7.43–7.54 (2H, *m*), 7.26–7.32 (2H, *m*), 7.09 (2H, *m*), 6.86–7.02 (2H, *m*), 5.71 (1H, *s*). ^{13}C NMR (DMSO-d_6, 500 MHz, δ, TMS = 0): 17.41 (CH), 57.44 (C), 117.3 (CN), 118.81 (Ar–C), 121.25 (Ar–C), 122.15 (Ar–C), 122.51 (Ar–C), 123.22 (Ar–C), 126.38 (Ar–C), 126.61 (Ar–C), 126.95 (Ar–C), 128.57 (Ar–C), 128.79 (Ar–C), 138.81 (Ar–C), 139.18 (Ar–C), 136.81 (Ar–C), 151.75 (Ar–C), 161.09 (C–NH_2). Anal. Calcd. for $C_{18}H_{12}N_2OS$: C, 71.03; H, 3.97; N, 9.20; S, 10.54; Found: C, 71.32; H, 3.66; N, 8.96; S, 10.76.

Xanthine oxidase assay

Bovine milk xanthine oxidase (grade 1, ammonium sulphate suspension, Sigma-Aldrich) activity was assayed spectrophotometrically by measuring the uric acid formation at 293 nm using a Hitachi U-3010 UV–visible spectrophotometer at 25 °C (Escribano *et al*., 1988; Takano *et al*., 2005). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 75 μM xanthine and 0.08 U of xanthine oxidase. Inhibition of xanthine oxidase activity by various inhibitors was measured by following the decrease in the uric acid formation at 293 nm at 25 °C. The enzyme was preincubated for 5 min, with test compound, dissolved in DMSO (1 % v/v), and the reaction was started by the addition of xanthine. Final concentration of DMSO (1 % v/v) did not interfere with the enzyme activity. All the experiments were performed in triplicate, and values were expressed as means of three experiments.

Molecular modelling study

The 3D structural coordinates of XO were obtained from protein databank (PDB ID: 1VDV) (Fukunari *et al*., 2004). The ligand structure was prepared in ChemDraw, and energy was minimized MM2 module of Chem3D ultra (ChemDraw Ultra 6.0 and Chem3D Ultra, 2000). The ligand was docked at the binding site using the GOLD 5.1 (GOLD, Evaluation Version 5.1 2012). Gold performs genetic algorithm-based ligand docking to optimize the conformation of ligand at the receptor binding site. GoldScore scoring function was used to find out the binding pose. GoldScore comprises four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals (vdw) energy, ligand internal vdw energy and ligand torsional strain energy.

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