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N-(1,3-Diaryl-3-oxopropyl)amides as a new template for xanthine oxidase inhibitors

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ABSTRACT

A series of forty two *N*-(1,3-diaryl-3-oxopropyl)amides were synthesized via an efficient, modified Dakin–West reaction and were evaluated for in vitro xanthine oxidase inhibitory activity for the first time. Structure–activity relationship analyses have been presented. Selected active xanthine oxidase inhibitors (**3r**, **3s**, and **3zh**) were assessed in vivo to study their anti-hyperuricemic effect in potassium oxonate induced hyperuricemic mice model. Compound **3s** emerged as the most potent xanthine oxidase inhibitor (IC_{50} = 2.45 μM) as well as the most potent anti-hyperuricemic agent. The basis of significant inhibition of xanthine oxidase by **3s** was rationalized by its molecular docking into catalytic site of xanthine oxidase.

1. Introduction

Purine substrates (hypoxanthine and xanthine) undergo catalytic oxidative hydroxylation to produce uric acid under the influence of xanthine oxidase (XO; EC 1.17.3.2) a versatile molybdoflavoprotein. During this process, XO generates superoxide anions and H_2O_2, which in the presence of chelated iron are converted into highly reactive hydroxyl radicals.[1–3] These reactive oxygen species (ROS) are associated in pathological events including inflammation, metabolic disorders, cellular aging, reperfusion damage, atherosclerosis, and carcinogenesis. ROS induce programmed cell death or necrosis, induce or suppress the expression of many genes, and activate cell signaling cascades. The oxidative damage of DNA in the development of certain cancers and lipid oxidative damage in the occurrence and progression of vascular disease are also associated with ROS.[2] There is an overwhelming acceptance that XO serum levels are increased in various pathological states like hepatitis, inflammation, ischemia-reperfusion, cancer and aging.[2,3] Thus the selective inhibition of XO may result in broad spectrum therapeutics for gout, cancer, inflammation and oxidative damage.[2–5] XO inhibitors can be broadly classified into purine and non-purine analogs on the basis of their structural similarities with the natural purines.[6] Various purine based compounds such as allopurinol,[3,4,6] 2-alkylhypoxanthines,[7] pterin and 6-formylpterin (pteridine analogs)[8] were developed as potent XO inhibitors (Fig. 1). However their significant effect on activities of purine and pyrimidine metabolism enzymes like guaninedeaminase, HGPRT (hypoxanthine-guanine phosphoribosyl transferase), PNP (purine nucleoside phosphorylase), OPRT (orotate phospho-ribosyl transferase) and OMPDC (orotidine-5-monophosphate decarboxylase)

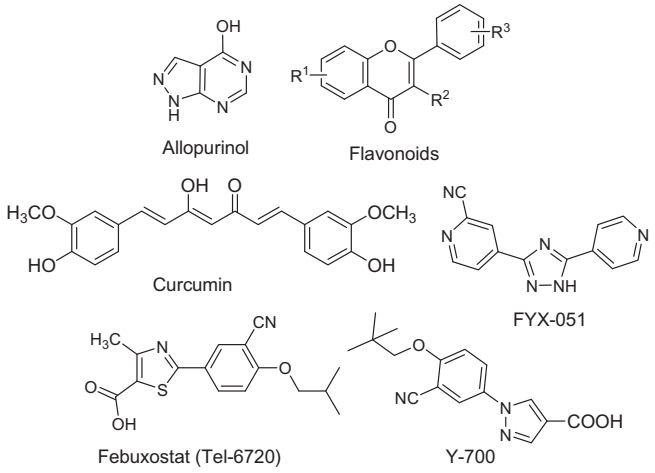

Figure 1. Some xanthine oxidase inhibitors.

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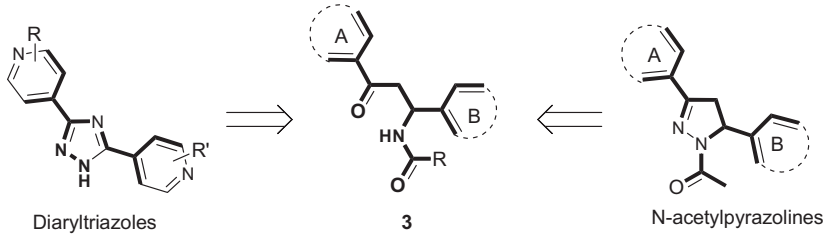


Figure 2. Design of target compounds (**3**).

and the reported hypersensitivity (Stevens–Johnsons) syndrome characterized by fever, skin rash, hepatitis, leukocytosis with eosinophilia and worsening renal function induced in some of the patients by allopurinol use[2–4] led to the search for XO inhibitors with structurally diverse and novel non-purine isosteres[9] such as febuxostat,[10] flavonoids,[11] FYX-051, a 1,3-diaryltriazole derivative[12] and curcumin[13] (Fig. 1).

Recently our research group reported the XO inhibitory potential of *N*-acetyl pyrazolines.[14] The rational designing of compounds as XO inhibitors derived from *N*-(1,3-diaryl-3-oxopropyl)amides (**3**) was thought of via chemical modification of both 1,3-diaryltriazoles and *N*-acetyl pyrazolines (Fig. 2) keeping in view their structural features such as two aromatic or heteroaromatic rings joined by a central linker, a site for hydroxylation near molybdenum metal, a carbamoyl group that might impart binding interaction(s) with amino acid(s) of the active site of enzyme and a non-purine based skeleton so as to exclude the possibility of target compounds being converted, like allopurinol, to unnatural nucleotides. Being successful in achieving the desired XO inhibitory activity in case of *N*-acetyl pyrazolines[14] where we replaced the central aromatic linker with non aromatic pyrazoline, its further replacement with an acylic β-acetamido carbonyl moiety is thought of. With this, we wish to report an efficient synthesis of *N*-(1,3-diaryl-3-oxopropyl)amides **3** and their biological evaluation as XO inhibitors for the first time.

2. Results and discussion

2.1. Optimization of reaction conditions for the synthesis of *N*-(1,3-diaryl-3-oxopropyl)amides

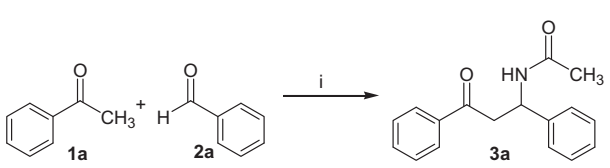
N-(1,3-Diaryl-3-oxopropyl)amides (**3**) are reported as the key intermediates for the synthesis of number of biologically and pharmaceutically important compounds.[15] They could easily be converted to 1,3-amino alcohols, which are precursors for the synthesis of several antibiotics.[16] *N*-(1,3-Diaryl-3-oxopropyl)amides have been recently found to be potent α-glucosidase inhibitors.[17]

While planning for the synthesis of **3**, it was realized that a convenient synthetic protocol is lacking. The synthesis of **3** has been documented either through acylation of β-amino ketones,[18] Michael addition of α,β-unsaturated ketones,[19] photoisomerization of phthalimides[20] or as a modified Dakin–West reaction[21] through multicomponent condensation of aryl aldehyde, enolizable ketone, acetyl chloride and acetonitrile or benzonitrile in the presence of Lewis acids[22] such as $CoCl_2$, Montmorillonite K-10 clay, $BiCl_3$ generated from BiOCl, $ZrOCl_2{\cdot}8H_2O$, heteropoly acids, $H_3PW_{12}O_{40}$, $Cu(OTf)_2$/$Sc(OTf)_3$, $FeCl_3$, ZnO, $K_5CoW_{12}O_{40}{\cdot}3H_2O$, Amberlyst-15, $CeCl_3{\cdot}7H_2O$, polyaniline supported $Co(OAc)_2$, I_2, $SiCl_4$–$ZnCl_2$, $Cu(BF_4)_2{\cdot}xH_2O$, TMSCl or protic/Brønsted acids[23] such as *p*-TsOH, $Fe(HSO_4)_3$, $NaHSO_4{\cdot}H_2O$, Nafion-H, NH_2SO_3H, H_2SO_4/SiO_2, $HClO_4/SiO_2$, and $Zr(HSO_4)_4/Mg(HSO_4)_4$. The existing methodologies suffer from one or other disadvantages such as prolonged reaction times, heating conditions, special efforts required to prepare the catalyst, need to

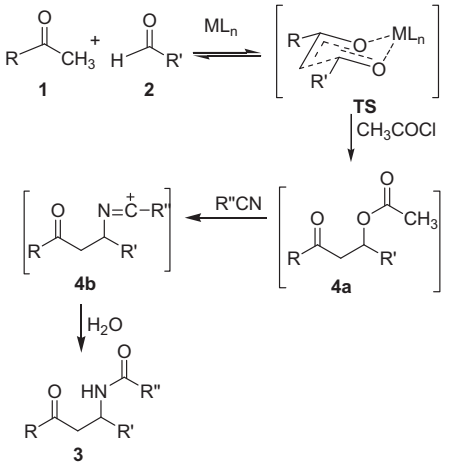
Table 1
Effect of solvent on one-pot synthesis of *N*-(3-oxo-1,3-diphenylpropyl)acetamide under the catalytic influence of various triflates and perchlorates[a]

Entry	Catalyst	Solvent	Amount	Time	Yield[b,c]
1	$LiClO_4$	Neat	100	30 h	59[d]
2	$Mg(ClO_4)_2$	Neat	1	45	81
3	$Ba(ClO_4)_2$	Neat	1	20	80
4	$Mn(ClO_4)_2$	Neat	1	45	75
5	$BiOClO_4{\cdot}6H_2O$	Neat	1	30	50
6	$Zn(ClO_4)_2{\cdot}6H_2O$	Neat	1	5	95
7	$Zn(ClO_4)_2{\cdot}6H_2O$	H_2O	1	2 h	66
8	$Zn(ClO_4)_2{\cdot}6H_2O$	EtOH	1	2 h	58[e]
9	$Zn(ClO_4)_2{\cdot}6H_2O$	DCM	1	2 h	40
10	$Zn(ClO_4)_2{\cdot}6H_2O$	Hexane	1	2 h	32
11	$Mg(OTf)_2$	Neat	1	25	81
12	$Zn(OTf)_2$	Neat	10	30 h	60[d]
13	$Cu(OTf)_2$	Neat	10	30 h	64[d]
14	$Bi(OTf)_3$	Neat	10	30 h	69[d]
15	$Sn(OTf)_2$	Neat	10	30 h	68[d]
16	$Sc(OTf)_3$	Neat	10	30 h	82[d]
17	$Yb(OTf)_3$	Neat	10	30 h	75[d]

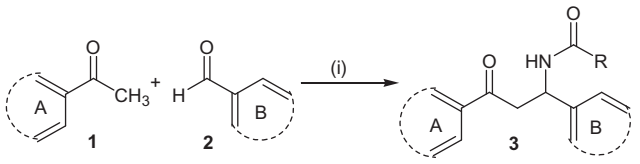
[a] A mixture of **1a** (2.5 mmol, 1 equiv), **2a** (1 equiv), acetyl chloride (1 equiv) and acetonitrile (1 equiv) was treated under the catalytic influence of various commercially available metal triflates and perchlorates at room temperature.
[b] Isolated yield of **3a**.
[c] A mixture of **1a** (1 equiv), **2a** (1 equiv) acetyl chloride (1 equiv) and acetonitrile (1 equiv) was taken.
[d] Reported yield and condition in Ref. 22f.
[e] 20% Claisen–Schmidt product was obtained.



Scheme 1. (i) Catalyst, acetonitrile, acetyl chloride, and solvent.



Scheme 2. Role of metal salts in catalyzing modified Dakin–West condensation.



Scheme 3. Synthesis of *N*-(1,3-diaryl-3-oxopropyl)amides. Reagents and conditions: (i) $Zn(ClO_4)_2{\cdot}6H_2O$ (1 mol %), acetonitrile or benzonitrile (1 equiv), acetyl chloride (1 equiv), rt, 1–20 min, 75–96%.

use excess reagents and catalyst, and tedious work-up procedures. Thus, there is necessity and scope to develop a convenient and an alternative methodology for the synthesis of the title compounds.

While designing a metal catalyst for the modified Dakin–West reaction, we considered the following aspects: (i) the catalyst should be derived from group I/II so as to reduce its cost, (ii) the central metal ion of the salt possess strong oxophilicity so that it could coordinate with both carbonyl oxygen of aldehyde as well as of ketone and, (iii) the counter ion should be highly electron withdrawing in nature capable to make central metal ion more oxophilic. The metals salts of strong protic acids such as triflic acid and perchloric acid were chosen for the study due to their pronounced oxophilic property. Recently metal perchlorates have been reported as highly electrophilic activation catalysts for conjugate addition, 1,1-diacetate formation, and in epoxide ring opening reactions.[24] Thus we planned to evaluate the catalytic efficiency of various commercially available metal perchlorates and triflates during modified Dakin–West reaction.

In a model reaction, a mixture of acetophenone **1a** (2.5 mmol, 1 equiv), benzaldehyde **2a** (1 equiv), acetyl chloride (1 equiv) and acetonitrile (1 equiv) was treated under the influence of various metal triflates and perchlorates to afford *N*-(3-oxo-1,3-diphenylpropyl)acetamide **3a** at room temperature (Scheme 1). The excellent results were obtained with $Zn(ClO_4)_2{\cdot}6H_2O$ (1 mol %) in carrying out reaction for 5 min (95% yield) at room temperature

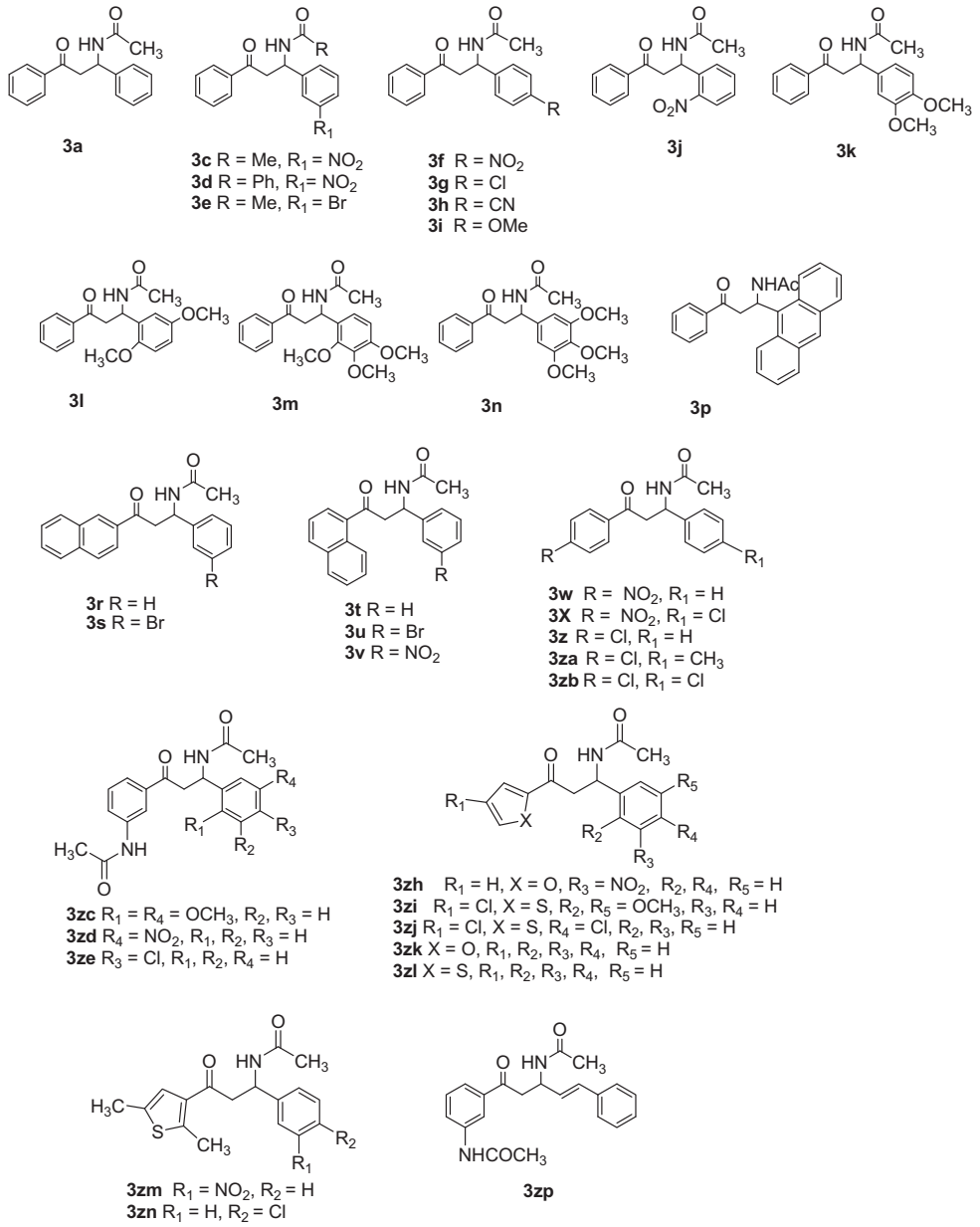


Figure 3. Synthesized *N*-(1,3-diaryl-3-oxopropyl)amides.

under neat conditions (Table 1). The formation of 20% Claisen–Schmidt product (entry 8) along with **3a** (58%) in carrying out the reaction in presence of ethanol prompted us to study the reaction without acetonitrile and acetyl chloride. However, no further enhancement in the yield of Claisen–Schmidt product occurred while carrying out the reaction of **1a** with **2a**, in presence of $Zn(ClO_4)_2{\cdot}6H_2O$ (1 mol %) for 12 h at room temperature in ethanol.

One can easily speculate that $Zn(ClO_4)_2{\cdot}6H_2O$ being an efficient electrophilic activation catalyst[22f] can coordinate with both carbonyl oxygen of ketone **1** and aldehyde **2** and result in formation of β-acetoxy carbonyl compound **2a** via a six-membered transition state **TS** (Scheme 2). The displacement of acetoxy group of **4a** may occur subsequently by nucleophilic nitrogen of the nitrile to provide a stable carbocation **4b**, which may further react in a Ritter fashion with water to afford **3**.

2.2. Synthesis of *N*-(1,3-diaryl-3-oxopropyl)amides

By using optimized procedure, it was possible to synthesize compounds **3a**–**3zp** (75–96%) via one-pot condensation of several aldehydes (**2a**–**2zp**) with aryl ketones (**1a**–**1zp**) under the catalytic influence of $Zn(ClO_4)_2{\cdot}6H_2O$ (1 mol %) at room temperature (Scheme 3, Fig. 3). The crude products were purified by column chromatography and were characterized by using spectroscopic techniques such as IR and NMR. The reaction was found to be compatible with diverse functional groups such as ether, nitro, cyano and halogens.

2.3. Biological evaluation of synthesized compounds for xanthine oxidase inhibitory activity

In vitro screening of the *N*-(1,3-diaryl-3-oxopropyl)amides (**3a–3zp**) using XO enzymatic assay was performed as described in the literature.[14,25] Each compound was tested in triplicate. Among the series of forty two compounds, three compounds **3r**, **3s**, and **3zh** were found to be most active against XO with IC_{50} ranging from 2.45 μM to 15.2 μM (Table 2). The inhibitory activity (IC_{50} = 2.45 μM) of the most potent compound **3s** was found to be comparable to that of allopurinol (IC_{50} = 8.3 μM), standard inhibitor of XO. Chemical structures of some known compounds (**3b**, **3o**, **3q**, **3y**, **3zf**, **3zg**, and **3zo**) with less potent XO inhibitory activity (IC_{50} >50 μM) are available in Supplementary data.

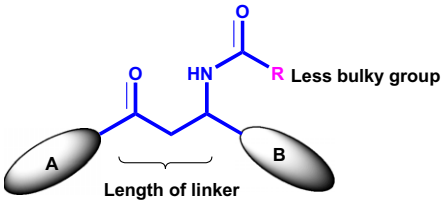
Table 2
Xanthine oxidase inhibitory assay values for compounds (**3a**–**3zp**)

Compounds	IC_{50}[a] (μM)
3a	38.3
3b	58.1
3c	26.2
3d	43.6
3e	28.1
3f	28.5
3g	27.1
3h	29
3i	45
3j	32.3
3k	44.3
3l	44
3m	46.1
3n	48.2
3o	53
3p	91.6
3q	59
3r	8.5
3s	2.45
3t	40
3u	42.2
3v	44
3w	48
3x	45.4
3y	52.6
3z	42
3za	44.6
3zb	41
3zc	43.2
3zd	32
3ze	41.8
3zf	63
3zg	65
3zh	15.2
3zi	25
3zj	22
3zk	26
3zl	27
3zm	19.8
3zn	21.7
3zo	78.3
3zp	69.2
Allopurinol	8.3

[a] Values are means of the three experiments.

2.4. Structure–activity relationship (SAR)

Some notions about structure–activity relationships emerged from these studies: (a) replacement of Ph on ring A with heterocycles such as furan or thiophene enhances the XO inhibitory activity (Table 2; compare **3a** with **3zk** and **3zl**), and the substitution of heterocycles with 2-naphthyl further potentiates the activity to the greater extent (compare **3a** with **3r**) which was not the case with 1-naphthyl (compare **3a** with **3t**), (b) in general, electronic factors such as introduction of deactivating group on ring A results in decrease in the inhibitory activity (compare **3a** with **3w** and **3z**) as compared to weak activating group (compare **3zo** with **3zp**), (c) change of Ph on ring B with either naphthyl, heteroaryl or anthranyl led to the decrease in the XO inhibitory activity which follow the order as Ph > 2-naphthyl > 1-naphthyl > heteroaryl > anthranyl (compare **3a** with **3o**, **3q**, **3zf**, **3zg**, and **3p**) which could be due to reason that increase in the steric hindrance disfavor the activity, (d) introduction of a deactivating group on ring B particularly at *m*-position resulted in enhancement of the activity (compare **3a** with **3c**, **3b** with **3d**, **3r** with **3s**, **3t** with **3u** and **3v** and **3zk** with **3zh**) as compared to activating groups, (e) substitution of Me of **3a** with bulkier Ph (**3b**) resulted in lowering of XO inhibitory activity and finally, and (f) lengthening of the linker between two rings results in dilution of the XO inhibitory activity which could be due to the increase in the flexibility of the molecules (compare **3a** with **3zo**). These observations can be depicted as shown below.



Ph < heteroaryl < naphthyl Ph > naphthyl > heteroaryl > Anthranyl

2.5. Evaluation of the effect of xanthine oxidase inhibitors in hyperuricemic mice model

Compounds **3s**, **3r**, and **3zh** were found to be active on the basis of in vitro experiment and were subjected to in vivo evaluation as

Table 3
Plasma uric acid concentration of mice after 3 h of treatment with potassium oxonate alone and incubation with inhibitors (allopurinol, **3s**, **3r**, and **3zh**)

Drug treatment	Dose (mg/kg)	Serum uric acid[a] (μM)
Control	–	3.01 ± 0.65
Potassium oxonate	300	7.80 ± 0.34[@@@]
Allo-10 + pot. oxonate	10	3.19 ± 0.21[***]
Allo-20 + pot. oxonate	20	2.93 ± 0.33[***]
Allo-50 + pot. oxonate	50	2.17 ± 0.42[***]
3s-10 + pot. oxonate	10	3.21 ± 0.43[***]
3s-20 + pot. oxonate	20	2.96 ± 0.17[***,$]
3s-50 + pot. oxonate	50	2.18 ± 0.13[***,#]
3r-10 + pot. oxonate	10	4.26 ± 0.67[***]
3r-20 + pot. oxonate	20	4.22 ± 0.89[***]
3r-50 + pot. oxonate	50	4.14 ± 0.44[***]
3zh-10 + pot. oxonate	10	7.13 ± 0.55
3zh-20 + pot. oxonate	20	7.01 ± 0.67
3zh-50 + pot. oxonate	50	6.98 ± 0.23

[a] Values are expressed as mean ± SD, (n = 6).
[@@@] p <0.001 versus control.
[***] p <0.001 versus potassium oxonate.
[$] p >0.05 versus Allo-20.
[#] p >0.05 versus Allo-50; [Allo-10, 20, and 50: Allopurinol 10, 20, and 50 mg/kg]; [**3s**-10, 20, and 50: **3s** 10, 20, and 50 mg/kg]; [**3r**-10, 20, and 50: **3r** 10, 20, and 50 mg/kg]; [**3zh**-10, 20, and 50: **3zh** 10, 20, and 50 mg/kg].

anti-hyperuricemic agents in potassium oxonate induced hyperuricemic mice model.[26,27] Induction with potassium oxonate 300 mg/kg caused significant (p <0.001) hyperurecemia in potassium oxonate control as compared to control. However, treatment with all the test drugs: allopurinol; and synthesized compounds; **3s** and **3r** at the doses 10, 20, and 50 mg/kg significantly (p <0.001) attenuated the potassium oxonate induced hyperurecemia as compared to potassium oxonate control (Table 3). The effect of **3s**-20 and 50 were significantly (p >0.05) different from the allo-20 and 50. This demonstrated that the compound **3s** as effective as the standard drug allopurinol.

The failure of **3r** and **3zh** to show dose-dependent response might have resulted due to: (a) ceiling effect-the submaximum possible effect by **3r** and **3zh** has already been achieved at the lowest dose and therefore the subsequent increase in the dose does not show direct correlation with the response and (b) the dose–response zone for the **3r** and **3zh** is much higher.

2.6. Molecular docking

In order to understand the binding conformation of *R* and *S* isomers of most potent XO inhibitor **3s**, its flexible molecular docking was carried out into the active site of XO using the GOLD software[28] assuming that it gets accommodated into the salicylic acid XO active site.[29] The Figure 4 shows the docking conformations of *R* and *S* isomers of **3s** (**3sR** and **3sS**) at the binding site of XO.

The binding site residues and overall binding mode of these compounds have been found similar to those observed with fabuxostat,[10] salicylic acid,[29] and curcumin.[13] The major interactions of **3sR** with XO include arene–arene interactions with Phe914 and Phe1009, two hydrogen bonds with Glu802 and Asn768 and two hydrophobic interactions with Thr803 and Leu1014 (Fig. 4b). The naphthalene ring of **3sR** is positioned in a cavity formed by Ser876, Arg880, Phe914, Phe1009, Thr1010, Val1011, and Ala1079 residues. The phenyl ring of residues Phe914 and Phe1009 lay parallel and perpendicular respectively to the plane of naphthalene ring of **3sR** (d = 3.78 Å and 3.50 Å). This arrangement of energetically favorable arene–arene interaction[30] has also been seen in the co-crystal structure of XO with salicylate and fabuxostat. This conservation argues for an important role in stabilizing the binding positions of aromatic substrates and that might well represent one of the key features of substrate recognition.[10,29] The backbone of **3sR** is stabilized by two H-bond and two hydrophobic interactions. The carbonyl function next to the naphthalene ring act as H-bond acceptor and involved in hydrogen bonding with carboxylate hydrogen of Glu802 residue. Similarly the carbonyl oxygen at amide function of inhibitor form hydrogen bond with the amide hydrogen of Asn768. The backbone CH_2 and free CH_3 groups of **3sR** show hydrophobic interactions with isopropyl group of Leu1014 and CH_3 group of Thr803 respectively. The phenyl group (ring B) of inhibitor is located in cavity formed by Lys771, Phe1013, and Met770. This phenyl group is perpendicular to the protonated amino group of Lys771 and likely to be involved in arene–cation interactions (Fig. 4b). The docking conformation of *S* isomer (**3sS**) and its interactions with residues are similar to those with the *R* isomer (Fig. 4a). This may be attributed to the free rotation by CH_2 linkage and stabilization of carbamoyl group ($NHCOCH_3$) and phenyl ring in **3sS** by Asn768, Thr803, and Lys771.

3. Conclusions

The present study was initiated with the aim of providing an efficient and convenient method for the synthesis of *N*-(1,3-diaryl-3-oxopropyl)amides. The optimization of the synthetic procedure was carried out and the effect of various metal perchlorates and triflates on the modified Dakin–West reaction was studied.

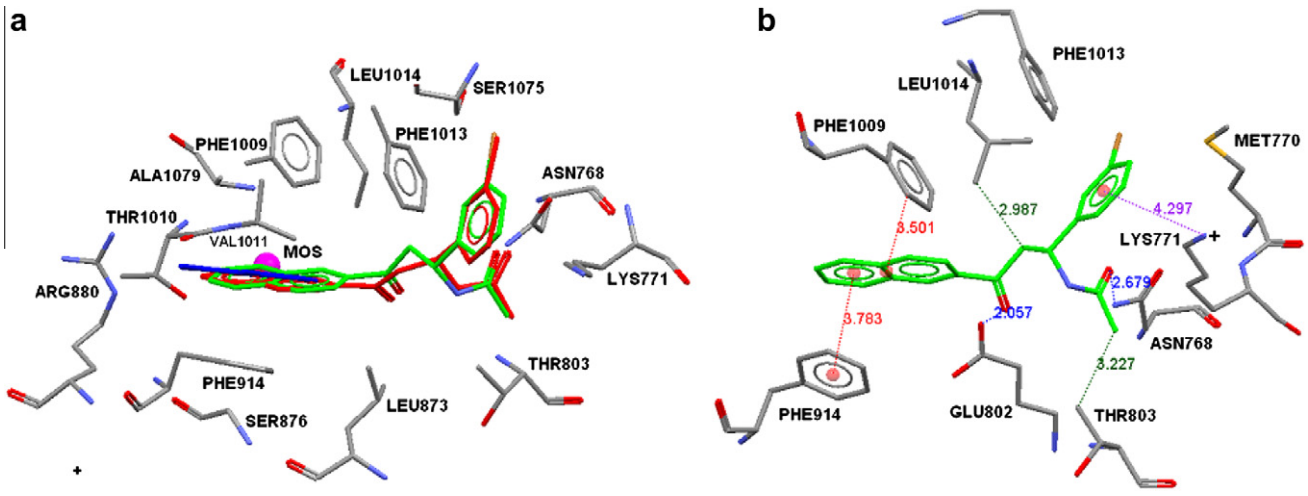


Figure 4. (a) Docking conformation of *R* and *S* isomers of **3s** at salicylic acid binding site of XO (*R* isomer: green, *S* isomer: red, salicylic acid: blue) and (b) binding interactions of *R* isomer with amino acid residue.

$Zn(ClO_4)_2{\cdot}6H_2O$ emerged as the best suitable catalyst for carrying out the modified Dakin–West condensation of aryl aldehydes with aryl ketones. Following the optimized procedure forty two *N*-(1,3-diaryl-3-oxopropyl)amides were synthesized out of which thirteen were found as new. The synthesized compounds were evaluated for in vitro XO inhibitory activity. SAR study revealed that: (i) nature of rings A and B, (ii) nature of substituent(s) on the rings A and B and (iii) the length of the linker greatly affect the XO inhibitory activity. Compound **3s** exhibited potent in vitro XO inhibitory as well as in vivo anti-hyperuricemic activities comparable to that of allopurinol. The docking study highlighted the role of amino acid residues involved in interactions with **3s** and the results are in agreement with the previous reported studies. Further lead modification is under progress and will be published due course of time. The compound **3s** is identified for further study.

4. Experimental

The reagents were purchased from Aldrich, Loba and CDH, India and used without further purification. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples and by spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR spectra). The NMR spectra were measured in $CDCl_3$ relative to TMS (0.00 ppm). IR (KBr pallets) spectra were recorded on a Fourier transform infrared (FT-IR) Thermo spectrophotometer. Melting points were determined in open capillaries and were uncorrected.

4.1. Typical experimental procedure for the synthesis of *N*-(3-oxo-1,3-diarylpropyl)acetamide and *N*-(3-oxo-1,3-diphenylpropyl)acetamide (3a)

To a magnetically stirred mixture of **2a** (2.5 mmol, 1 equiv, 0.26 g), **1a** (1 equiv, 0.3 g), acetyl chloride (1 equiv) and acetonitrile (1 equiv) was added $Zn(ClO_4)_2{\cdot}6H_2O$[31] (9.3 mg, 0.025 mmol, 1 mol %) at room temperature. The progress of the reaction was monitored by TLC (5 min). After the completion of reaction, the mixture was poured into 50 mL ice-water. The solid product was filtered, washed with ice-water, dried and purified via column chromatography (silica gel 60–120 #) using EtOAc–hexane as eluent to afford pure white solid **3a** (0.63 g, 95%). Mp 103–105 °C (lit.[22o] 102–104 °C).

The remaining reactions were carried out following the general procedure. In each occasion, the spectral data (IR, ^{1}H NMR and ^{13}C NMR) of known compounds such as *N*-(3-oxo-1,3-diphenylpropyl) benzamide[23f] (**3b**), *N*-[1-(2-nitrophenyl)-3-oxo-3-phenylpropyl] acetamide[22d] (**3c**), *N*-[1-(3-nitrophenyl)-3-oxo-3-phenylpropyl] acetamide[22d] (**3d**), *N*-[1-(3-nitrophenyl)-3-oxo-3-phenylpropyl]-benzamide[22d] (**3e**), *N*-[1-(4-nitrophenyl)-3-oxo-3-phenylpropyl] acetamide[22d] (**3f**), *N*-[1-(4-chlorophenyl)-3-oxo-3-phenylpropyl] acetamide[22m] (**3g**), *N*-[1-(3-bromophenyl)-3-oxo-3-phenylpropyl] acetamide[22f] (**3h**), *N*-[1-(4-cyanophenyl)-3-oxo-3-phenylpropyl] acetamide[22i] (**3i**), *N*-[1-(4-methoxyphenyl)-3-oxo-3 phenylpropyl] acetamide[22m] (**3j**), *N*-[1-(3,4-dimethoxyphenyl)-3-oxo-3-phenylpropyl]acetamide[22g] (**3k**), *N*-[1-(2,5-dimethoxyphenyl)-3-oxo-3-phenylpropyl]acetamide[23f] (**3l**), *N*-[1-(2,3,4-trimethoxyphenyl)-3-oxo-3-phenylpropyl]acetamide[22i] (**3m**), *N*-[1-(3,4,5-trimethoxyphenyl)-3-oxo-3-phenylpropyl]acetamide[22g] (**3n**), *N*-(1-naphthalen-2-yl-3-oxo-3-phenylpropyl)acetamide[22m] (**3o**), *N*-[1-(2-oxo-2-phenylethyl)-3-phenylallyl]acetamide[23f] (**3q**) *N*-[3-(4-Nitro-phenyl)-3-oxo-1-phenyl-propyl]-acetamide[23h] (**3zd**), *N*-[1-(4-chloro-phenyl)-3-(4-nitro-phenyl)-3-oxo-propyl]-acetamide[23h] (**3ze**), *N*-[1-(4-methoxy-phenyl)-3-(4-nitro-phenyl)-3-oxo-propyl]-acetamide[23h] (**3zf**), *N*-[3-(4-chloro-phenyl)-3-oxo-1-phenyl-propyl]-acetamide[23i] (**3zg**), *N*-[3-(4-chloro-phenyl)-3-oxo-1-*p*-tolyl-propyl]-acetamide[23i] (**3zh**), *N*-[1,3-bis-(4-chloro-phenyl)-3-oxo-propyl]-acetamide (**3zi**)[23i] were found to be identical with those reported in the literature. The physical data of 13 new compounds are provided below.

4.1.1. *N*-(1-Anthracen-9-yl-3-oxo-3-phenylpropyl)-acetamide (3p)

White solid, yield 75%, mp 120–122 °C. IR (KBr): 3260, 1672, 1645, 1601, 1592 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.05 (s, 3H), 4.01 (dd, J_{12} = 7.6, J_{13} = 16.1 Hz, 1H), 4.22 (dd, J_{12} = 5.4, J_{13} = 16.1 Hz, 1H), 6.40 (m, 1H), 7.18 (m, D_2O exchangeable NH), 7.27–7.61 (m, 7H), 7.91–7.93 (m, 4H), 8.03–8.04 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.4, 169.8, 136.6, 133.0, 131.8, 129.8, 128.8, 128.5, 128.2, 126.5, 124.9, 124.2, 45.8, 45.1, 23.34. Anal. Calcd for $C_{25}H_{21}NO_2$: C, 81.72; H, 5.76; N, 3.81. Found: C, 81.89; H, 6.02; N, 3.98.

4.1.2. *N*-[1-(3-Bromophenyl)-3-naphthalen-2-yl-3-oxo-propyl]acetamide (3s)

White solid, yield 85%, mp 133–135 °C. IR (KBr): 3273, 1682, 1638, 1595, 1560, 1371, 819 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.05 (s, 3H), 3.73 (dd, J_{12} = 5.7, J_{13} = 17.1 Hz, 1H), 3.90 (dd, J_{12} = 5.1, J_{13} = 17.1 Hz, 1H), 5.60 (m, 1H), 6.82 (d, J = 8.2 Hz, D_2O exchangeable NH), 7.17 (m, 1H), 7.30–7.65 (m, 5H), 7.87–7.97 (m, 4H), 8.43 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.2, 169.5, 143.4, 135.8, 133.7, 132.4, 130.5, 130.2, 129.6, 129.5, 128.9, 128.7, 127.8, 127.0, 125.2, 123.4, 122.8, 49.4, 42.8, 23.4. Anal. Calcd for $C_{21}H_{18}BrNO_2$: C, 63.65; H, 4.58; N, 3.53. Found: C, 63.73; H, 4.82; N, 3.82.

4.1.3. *N*-[1-(3-Bromophenyl)-3-naphthalen-1-yl-3-oxopropyl]-acetamide (3u)

White solid, yield 78%, mp 124–125 °C. IR (KBr): 3265, 1677, 1648, 1607, 1595, 773 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.06 (s, 3H), 3.51(dd, J_{12} = 5.8, J_{13} = 16.7 Hz, 1H), 3.79 (dd, J_{12} = 5.8, J_{13} = 16.7 Hz, 1H), 5.56 (m, 1H), 6.82 (d, J = 8.2 Hz, D_2O exchangeable NH), 7.22–7.51 (m, 3H), 7.55 (m, 4H), 7.74 (m, 1H), 7.83 (m, 1H), 8.02 (d, J = 8.2 Hz, 1H), 8.42 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.5, 169.5, 143.3, 135.1, 133.9, 133.4, 130.6, 130.2, 129.9, 129.6, 128.5, 128.2, 128.0, 126.6, 125.3, 125.2, 124.3, 122.8, 49.9, 46.1, 23.4. Anal. Calcd for $C_{21}H_{18}BrNO_2$: C, 63.65; H, 4.58; N, 3.53. Found: C, 63.75; H, 4.82; N, 3.46.

4.1.4. *N*-[3-Naphthalen-1-yl-1-(3-nitrophenyl)-3-oxo-propyl]-acetamide (3v)

White solid, yield 91%, mp 135–136 °C. IR (KBr): 3393, 1678, 1658, 1555, 1537, 1359, 1143 cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$) : δ = 2.01 (s, 3H), 3.49 (dd, J_{12} = 5.7, J_{13} = 17.1 Hz, 1H), 3.83 (dd, J_{12} = 5.4, J_{13} = 17.1 Hz, 1H), 5.57 (dd, J_{12} = 5.7, J_{13} = 13.5 Hz, 1H), 7.03 (s, D_2O exchangeable NH), 7.13 (t, J = 7.8 Hz, 1H), 7.25–7.34 (m, 2H), 7.52–7.62 (m, 3H), 7.82–7.93 (m, 4H), 8.39 (1H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 197.97, 169.64, 143.53, 135.66, 133.64, 132.29, 130.42, 130.12, 130.08, 129.57, 129.55, 128.78, 128.58, 127.72, 126.92, 125.20, 123.39, 122.68, 49.36, 43.05, 23.32. Anal. Calcd for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 70.0; H, 5.21; N, 7.62.

4.1.5. *N*-[3-Furan-2-yl-1-(3-nitrophenyl)-3-oxo-propyl]-acetamide (3zh)

White solid, yield 77%, mp 132–133 °C. IR (KBr): 3262, 1671, 1641, 1602, 1590, 1340, 1230 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.06 (s, 3H), 3.34 (dd, J_{12} = 5.4, J_{13} = 16.7 Hz, 1H), 3.59 (dd, J_{12} = 5.76, J_{13} = 16.7 Hz, 1H), 5.61 (m, 1H), 6.54 (m, D_2O exchangeable NH), 6.98 (d, J = 7.8 Hz, 1H), 7.71 (m, 4H), 8.07–8.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.7, 169.7, 152.2, 148.4, 147.1, 143.3, 132.8, 129.5, 122.4, 121.5, 118.2, 112.7, 49.2, 42.6, 23.3. Anal. Calcd for $C_{15}H_{14}N_2O_5$: C, 59.60; H, 4.67; N, 9.27. Found: C, 59.79; H, 4.82; N, 9.99.

4.1.6. *N*-{1-[2-(3-Acetylaminophenyl)-2-oxo-ethyl]-3-phenyl-allyl}-acetamide (3zp)

Brown solid, yield 76%, mp 70–71 °C. IR (KBr): 3373, 1685, 1650, 1587, 1137 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.07 (s, 3H), 2.18(s, 3H), 3.28 (m, 1H), 3.47 (m, 1H), 5.12 (m, 1H), 6.30–6.93 (m, 3H), 7.02–7.74 (m, 6H), 7.88–8.21 (s, 3H), 9.73 (s, D_2O exchangeable NH). Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 72.11; H, 6.70; N, 7.82.

4.1.7. *N*-[3-(3-Acetylaminophenyl)-1-(2,5-dimethoxy-phenyl)-3-oxo-propyl]-acetamide (3zc)

Brown solid, yield 77%, mp 90–91 °C. I.R (KBr): 3363, 1676, 1659, 1563, 1143 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) : δ = 2.06 (s, 3H), 2.13 (s, 3H), 3.39 (m, 1H), 3.71 (m, 1H), 3.80 (s, 6H), 5.72 (m, 1H), 6.87 (s, D_2O exchangeable NH), 7.16–7.26 (m, 3H), 7.58–7.68 (m, 3H), 8.01 (d, J = 8.1 Hz, 1H), 9.73 (s, D_2O exchangeable NH). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 191.04, 170.54, 150.58, 136.78, 129.42, 124.89, 125.56, 123.98, 123.52, 119.64, 114.96, 112.76, 111.66, 55.86, 48.03, 43.49, 24.70, 22.76. Anal. Calcd for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.34; H, 6.43; N, 7.33.

4.1.8. *N*-[3-(3-Acetylaminophenyl)-1-(3-nitro-phenyl)-3-oxo-propyl]-acetamide (3zd)

Brown solid, yield 93%, mp 91–92 °C. IR (KBr): 3336, 1686, 1651, 1565, 1530, 1387, 1132 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.97 (s, 3H), 2.14 (s, 3H), 3.44–3.65 (m, 2H), 5.63 (m, 1H), 7.36 (s, D_2O exchangeable NH), 7.50–8.07 (m, 4H), 8.22–8.42 (m, 4H), 9.79 (s, D_2O exchangeable NH). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 196.18, 147.96, 144.71, 139.71, 136.58, 133.39, 129.21, 128.81, 124.22, 122.61, 121.80, 121.46, 118.86, 48.79, 44.15, 24.06, 22.82. Anal. Calcd for $C_{19}H_{19}N_3O_5$: C, 61.78; H, 5.18; N, 11.38. Found: C, 62.02; H, 5.33; N, 11.22.

4.1.9. *N*-[3-(3-Acetylaminophenyl)-1-(4-chloro-phenyl)-3-oxo-propyl]-acetamide (3ze)

White solid, yield 92%, mp 160–161 °C. IR (KBr): 3343, 1681, 1648, 1573, 1143, 670 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.03 (s, 3H), 2.19 (s, 3H), 3.39 (dd, J_{12} = 6.3, J_{13} = 17.1 Hz, 1H), 3.71 (dd, J_{12} = 5.4, J_{13} = 17.14 Hz, 1H), 5.53 (m, 1H), 6.68 (d, J = 7.5 Hz, D_2O exchangeable NH), 7.35–7.45 (m, 3H), 7.54–7.68 (m, 2H), 7.79 (d, J = 8.1 Hz, 1H), 8.01 (2H, m), 9.81 (s, D_2O exchangeable NH). Anal. Calcd for $C_{19}H_{19}ClN_2O_3$: C, 63.60; H, 5.34; N, 7.81. Found: C, 63.99; H, 5.47; N, 8.12.

4.1.10. *N*-[3-(4-Chlorothiophen-2-yl)-1-(2,5-dimethoxy-phenyl)-3-oxo-propyl]-acetamide (3zi)

Dark brown solid, yield 79%, mp 102–103 °C, IR (KBr): 3387, 1686, 1661, 1567, 1183 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.01 (s, 3H), 3.24 (m, 1H), 3.55 (m, 1H), 3.79 (s, 6H), 5.46 (m, 1H), 6.79–6.94 (4H, m), 7.26 (m, 1H), 7.49 (s, 1H). Anal. Calcd for $C_{17}H_{18}ClNO_4S$: C, 55.51; H, 4.93; N, 3.81; S, 8.72. Found: C, 55.33; H, 5.10; N, 3.88; S, 9.0.

4.1.11. *N*-[1-(4-Chlorophenyl)-3-(4-chloro-thiophen-3-yl)-3-oxo-propyl]-acetamide (3zj)

Light brown solid, yield 95%, mp 102–103 °C, IR (KBr): 3297, 1682, 1653, 1565, 1132, 680 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.01 (s, 3H), 3.24 (dd, J_{12} = 17.1, J_{13} = 5.7 Hz, 1H), 3.55 (dd, J_{12} = 5.4, J_{13} = 17.1 Hz, 1H), 5.46 (m, 1H), 6.84 (s, D_2O exchangeable NH), 6.94 (s, 1H), 7.26–7.39 (m, 4H), 7.49 (s, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 190.19, 169.65, 142.14, 140.79, 138.96, 133.31, 132.37, 128.78, 127.80, 49.61, 42.90, 23.38. Anal. Calcd for $C_{15}H_{13}Cl_2NO_2S$: C, 52.64; H, 3.83; N, 4.09; S, 9.37. Found: C, 3.24; H, 3.92; N, 3.58; S, 9.55.

4.1.12. *N*-[3-(2,5-Dimethylthiophen-3-yl)-1-(3-nitro-phenyl)-3-oxo-propyl]-acetamide (3zm)

Yellow solid, yield 90%, mp 126–127 °C. IR (KBr): 3363, 1687, 1651, 1565, 1530, 1378, 1147, 697 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) : δ = 2.07 (s, 3H), 2.38 (s, 3H), 2.59 (s, 3H), 3.31 (m, 1H), 3.54 (m, 1H), 5.57 (m, 1H), 6.94 (s, D_2O exchangeable proton), 7.15 (m, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.69 (d, J = 6.94 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 8.19 (s, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 193.71, 169.77, 149.08, 148.29, 143.64, 135.78, 134.71, 132.92, 129.42, 125.50, 122.21, 121.28, 49.13, 45.37, 23.40, 16.13, 14.91. Anal. Calcd for $C_{17}H_{18}N_2O_4S$: C, 58.94; H, 5.24; N, 8.09; S, 9.26. Found: C, 59.11; H, 5.42; N, 8.38; S, 9.47.

4.1.13. *N*-[1-(4-Chlorophenyl)-3-(2,5-dimethyl-thiophen-3-yl)-3-oxo-propyl]-acetamide (3zn)

Light brown solid, yield 85%, mp 87–88 °C. IR (KBr): 3372, 2952, 1682, 1649, 1585, 1545, 1132, 680 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.03 (s, 3H), 2.37 (s, 3H), 2.58 (s, 3H), 3.22 (m, 1H), 3.46 (m, 1H), 5.44 (m, 1H), 6.93 (s, D_2O exchangeable NH), 7.08–7.48 (m, 4H), 7.97 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 193.91, 170.60, 148.74, 139.52, 135.68, 134.94, 133.15, 131.35, 128.74, 127.94, 125.70, 49.60, 45.75, 22.98, 22.67, 16.15, 14.97. Anal. Calcd for $C_{17}H_{18}ClNO_2S$: C, 60.80; H, 5.40; N, 4.17; S, 9.55. Found: C, 60.92; H, 5.61; N, 3.89; S, 9.71.

4.2. Xanthine oxidase assay

Bovine milk xanthine oxidase (grade 1, ammonium sulfate suspension, Sigma–Aldrich) activity was assayed spectrophotometrically by measuring the uric acid formation at 293 nm[14,25] using a Hitachi U-3010 UV–visible spectrophotometer at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 75 μM xanthine and 0.08 units 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.

4.3. Evaluation of the effect of xanthine oxidase inhibitors in hyperuricemic mice model

4.3.1. Animals and treatment

Male Swiss mice (25–30 g) were taken from central animal facility (CAF), NIPER, S.A.S. Nagar, Punjab. Animals were housed in a room at controlled temperature of 22 ± 1 °C with 12 h-light/12 h-dark cycle and allowed free access to food and water. In order to cause hyperuricemic status, potassium oxonate 300 mg/kg was quickly and carefully injected to the intraperitoneal (i.p.) cavity of mice without anesthesia.[27] The test compound or allopurinol (10, 20, and 50 mg/kg), was dissolved in 1% DMSO and then administered (intraperitoneal, i.p.) to the mice, 1 h after the potassium oxonate injection. Blood was collected from the mice via tail tip cuts, 2 h after the administration of the test compound (**3s**, **3r**, and **3zh**) or allopurinol. All mice were sacrificed after the blood collection. This experimental design was approved by the Institutional Animal Ethics Committee (IAEC), NIPER.

4.3.2. Measurement of plasma uric acid concentration

The uric acid in mice serum was measured by HPLC (Shimadzu, Japan) according to the reported method[26] with slight modifications as described. The blood sample was collected from the tail

tip of the treated mice and allowed to clot at room temperature. After clotting, the blood sample was centrifuged at 7000×*g* for 10 min to collect the serum. Serum was collected in separate tubes and treated with an equivalent volume of perchloric acid (0.1 mM). Different serum samples were then vortexed and centrifuged again at 2000×*g* for 5 min to precipitate out undesired proteins. Supernatant was collected in the separate tube and diluted with running buffer (50 mM dihydrogen potassium phosphate, pH 4.0) and filtered through 0.2 μm filter. The clear filtrate obtained was used for the HPLC assay.[26] A model 10A high-performance liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a UV spectrophotometric detector set at 293 nm was used. The quantitative HPLC separations were performed at 25 °C on a Waters C-18 reversed-phase column (250 × 4.60 mm, 5 μm particle size). The injection volume was 10 μl, the mobile phase employed consisted of 90% dihydrogen potassium phosphate buffer (50 mM, pH 4) and 10% acetonitrile. The flow rate was 0.4 mL/min and the absorbance detector was set at 293 nm.

4.4. Molecular modeling

The coordinates of bovine milk XO complexed with salicylic acid were obtained from protein data bank (PDB entry: 1fiq).[29] The ligands were drawn in ChemDraw and subjected to energy minimization in the MOPAC module, using the AM1 procedure for closed shell systems, implemented in the CS Chem3D Ultra.[32] The ligands were docked in to the active site of XO using the GOLD 4.0.1 (Cambridge Crystallographic Data Center, Cambridge, UK)[28] Gold performs genetic algorithm based ligand docking to optimize the conformation of ligand at the receptor binding site. It utilizes GoldScore fitness function to evaluate the various conformations of ligand at the binding site and comprises four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals (vdw) energy, ligand internal vdw energy, and ligand torsional strain energy. Each isomer was docked 10 times and each pose was ranked according to its GoldScore fitness function. The conformations with highest score were selected for discussion.[33]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.039.

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