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Isocytosine-based inhibitors of xanthine oxidase: Design, synthesis, SAR, PK and in vivo efficacy in rat model of hyperuricemia

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ABSTRACT

Structure–activity relationship studies were carried out for lead generation following structure-guided design approach from an isocytosine scaffold identified earlier for xanthine oxidase inhibition. A 470-fold improvement in in vitro IC_{50} was obtained in the process. Five most potent compounds with nanomolar IC_{50} values were selected for pharmacokinetics and in vivo experiments. The best compound showed good in vivo activity when administered intraperitoneally but was not active by oral route. The results suggest that improvement in oral exposure could improve the in vivo efficacy of this series.

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Hyperuricemia is a metabolic disorder characterized by elevated levels of serum uric acid (sUA). It is often associated with diabetes, hypertension, and cardiovascular problems and is considered as a high risk factor for gout and oxidative stress.[1]

Hyperuricemia is caused by dietary, environmental and genetic factors[2] with two main lines of treatment[3] being reducing production and increasing excretion of uric acid (UA). The former is achieved by administration of xanthine oxidase (XO) inhibitors (XOIs) and the latter by uricosuric agents. XO is the key enzyme in purine metabolism to UA in the last two steps of the pathway.[4] Currently used XOIs for treatment of hyperuricemia are allopurinol[5] and febuxostat[6,7] (Fig. 1). They are associated with an adverse event profile that includes gastrointestinal, hepatic, renal and hematological toxicities, skin problems and allergic reactions.

We recently identified compound **1** (Fig. 1), a novel isocytosine based scaffold from our in-house corporate collection through virtual screening and testing in enzymatic assay.[8a] It showed an IC_{50} of 9.4 μM in XO inhibition assay and 84% reduction in sUA level in potassium oxonate-induced hyperuricemic rat model. Docking to XO showed that keto/enol group in isocytosine formed H-bonds with Arg880 and Thr1010 and $-NH_2$ with Glu802. Limited structure–activity relationship (SAR) suggested that these are essential interactions, which probably serve the role of anchoring the molecules in the active site. Docking studies suggested possible directions for modifications that could improve in vitro potency of the series.[8a] Here, we present our hit-to-lead efforts by repeated cycles of structure-guided design, synthesis and in vitro screening in XO inhibition assay,[8b] resulting in compounds with nanomolar activity. Selected active compounds were tested in vivo in hyperuricemic rat model,[8c] revealing 2 compounds with good efficacy.

Analysis of docking (PDB code 1VDV)[9] of the novel hit revealed that R points towards the solvent accessible region at the entrance of the pocket, which is lined by many hydrophobic and a few polar residues. SAR was obtained by diverse substitutions at R (Table 1), docked using GLIDE[10,11] synthesized (Scheme 1) and tested in vitro.[8b]

The observed in vitro activities corresponded well with predictions made by docking studies (Table 1) using docking scores,

Abbreviations: XO, xanthine oxidase; XOI, xanthine oxidase inhibitor; PK, pharmacokinetics; AUC, area under curve; po, per oral; ip, intraperitoneal; SAR, structure–activity relationship; UA, uric acid; sUA, serum UA.

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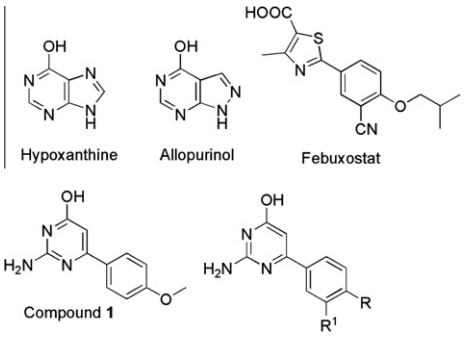
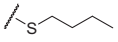
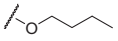
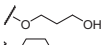


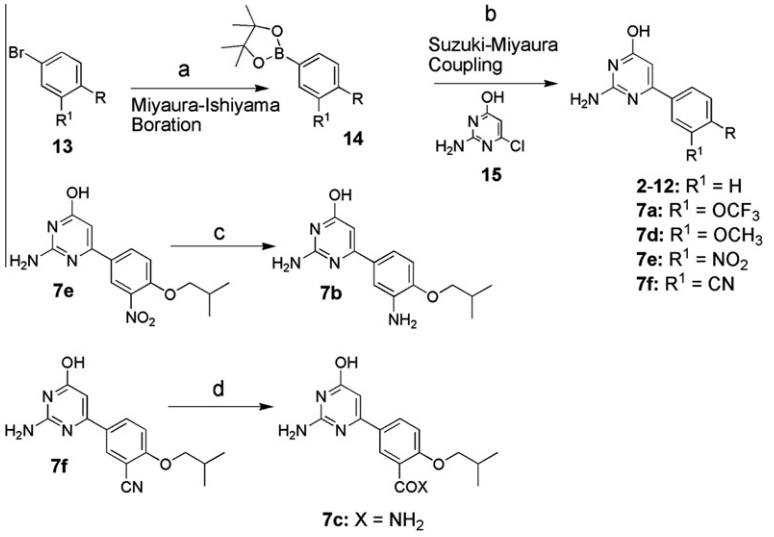
Figure 1. Structures of hypoxanthine, allopurinol, febuxostat and compound **1**. Potential sites of modifications on the phenylisocytosine scaffold are also shown.

docked poses and essential interactions with active site residues. Small and polar –OH group (**2**) docked in the correct pose with good score, but lost hydrophobic interactions with nonpolar residues at the entrance of the pocket and was inactive. Branched aliphatic chain (**3**) showed good activity. Branched and linear chains with thioether or ether linker (**4**, **5**, **6**, **7**, **8**) had similar scores and gave significant improvement in activity, probably due to improved electrostatic interactions with Asn768 and Lys771. Isobutoxy group (**7**, Fig. 2) resulted in one of the most potent compounds from this set with an IC_{50} value of 0.6 μM. Saturated cyclic rings with polarity such as morpholine (**9**) docked well but did not improve IC_{50} values, perhaps due to badly placed Ns in hydrophobic region of protein. However, aromatic phenyl ring (**10**) boosted the activity by 30-fold from 9.4 μM to 0.3 μM. This may be attributed to π-stacking interactions of the terminal aromatic ring with Phe649 and Phe1013 at the entrance to the pocket. Introducing a linker between the two phenyl rings improved dock scores but did not translate to improvement in potency (**11**, **12**).

Plasma pharmacokinetics (PK) of **7** was studied in Wistar/Sprague Dawley rats at a single 100 mg/kg dose by oral route (po) over

Table 1
Analogs of compound **1** with modifications at R

Compound ID	R	Yields (%)	Docking scores kcal/mol	In vitro IC_{50} μM
2	–OH	49.18	−9.77	>30
3		54.90	−10.80	4.95 ± 0.19
4		32.27	−10.75	1.63 ± 0.08
5		87.66	−10.51	2.44 ± 0.20
6		46.38	−10.15	2.10 ± 0.19
7		49.94	−9.84	0.60 ± 0.07
8		19.55	−10.04	9.24 ± 0.17
9		7.66	−10.25	16.52 ± 3.05
10		39.80	−9.08	0.31 ± 0.08
11		34.70	−11.18	3.65 ± 0.79
12		40.89	−10.70	2.47 ± 1.43
Febuxostat			−12.05	0.03 ± 0.00
Allopurinol			−8.47	4.19 ± 52



Scheme 1. Synthetic scheme for compounds in Tables 1 and 3: Reagents and conditions: (a) $Pd(dppf)Cl_2.CH_2Cl_2$, dioxan, bis-pinacolato diborane, potassium acetate,120 °C, 8 h. (b) $Pd(PPh_3)_4$, DMF, Na_2CO_3, microwave, 140 °C, 1–2 h. (c) Raney-Ni /H_2, 40 psi, DMF. (d) NaOH, MeOH, 140 °C, 12 h.

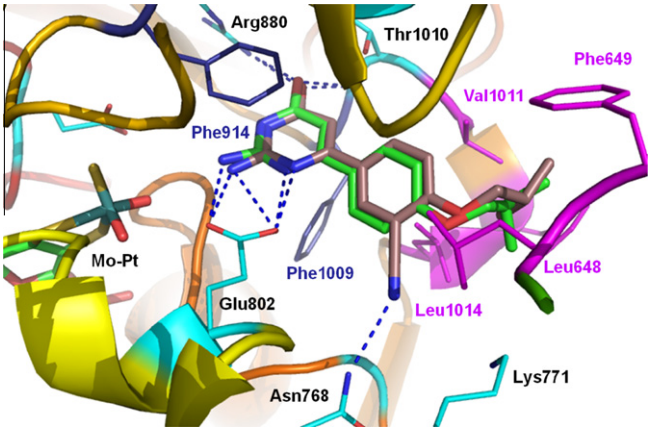


Fig. 2. Docked poses of compounds **7** (green) and **7f** (brown). Compound **7f** forms additional H-bond with Asn768 which adds to its potency. This figure has been generated in Pymol.[12]

Table 2
Pharmacokinetic parameters of compounds **1**, **7** and **10**

PK Parameters	**1** (100 mg/kg, po)	**7** (100 mg/kg, po)	**10** (100 mg/kg, po)	**10** (50 mg/kg, ip)
T_{max} (h)	0.25	0.5	0.88	3.33
C_{max} (μg/ml)	5.1	1.31	0.21	1.88
C_{max} (μM)	23.48	5.16	0.81	7.13
AUC_{last} (h∗μg/ml)	4.08	4.39	0.6	21.92
$AUC_{0\text{-infinity}}$ (h∗μg/ml)	4.11	5.68	0.89	23.14
Half-life (h)	6.59	3.42	2.85	5.52

Table 3
Analogs of compound **7** with modifications at R^1

Compound ID	R^1	Yields (%)	Docking scores kcal/mol	In vitro IC_{50} μM
7a	OCF_3	20.90	−9.96	>20
7b	NH_2	50.00	−10.33	6.72 ± 0.09
7c	$CONH_2$	2.57	−11.35	1.92 ± 0.7
7d	OCH_3	35.87	−10.53	0.95 ± 0.65
7e	NO_2	35.88	−10.33	0.14 ± 0.03
7f	CN	19.11	−10.98	0.02 ± 0.00
Febuxostat			−12.05	0.03 ± 0.00
Allopurinol			−8.47	5.77 ± 0.42

Table 4
Pharmacokinetic parameters of compounds **7d**, **7e** and **7f** in rats

PK parameters	**7d**(50 mg/kg ip)	**7e**(50 mg/kg ip)	**7f**(10 mg/kg po)	**7f**(2 mg/kg iv)
T_{max} (h)	2	2	0.19	0.03
C_{max} (μg/ml)	9.91	2.92	0.07	1.19
C_{max} (μM)	34.27	9.59	0.23	4.19
AUC_{last} (h∗μg/ml)	27.6	13.98	0.2	0.47
$AUC_{0\text{-infinity}}$ (h∗μg/ml)	27.72	17.55	0.31	0.48
Half-life (h)	0.85	12.25	11.45	0.63
%F (AUC_{last})	—	—	8.6	NA[a]
%F ($AUC_{0\text{-infinity}}$)	—	—	12.7	NA
C_O (ng/ml)	—	—	NA	1360.78
Vd (mg/kg)	—	—	NA	3825.58
CL (ml/h/kg)	—	—	NA	4279.22

[a] NA: not applicable.

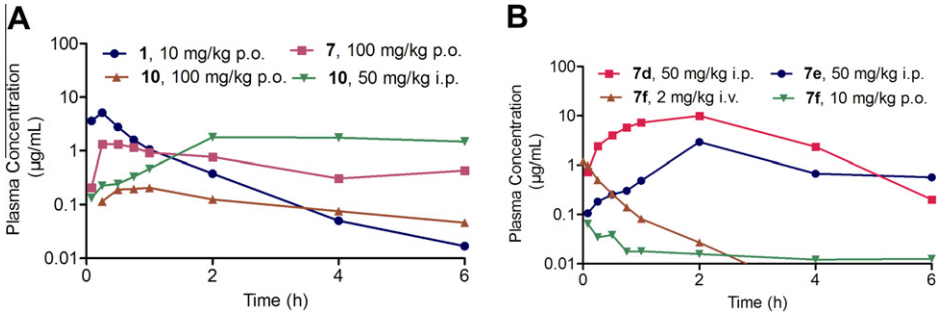


Figure 3. Plasma concentration-time profiles of compounds (A) **1**, **7** and **10** (B) **7d**, **7e** and **7f** in rats.

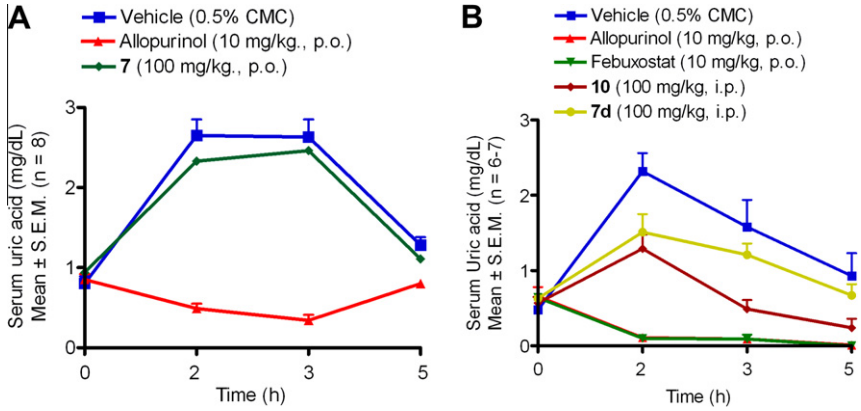


Figure 4. Time course of response of sUA levels for different treatments in hyperuricemic rats. Antihyperuricemic effect of (A) orally administered **7** (B) intraperitoneally administered **10** and **7d**.

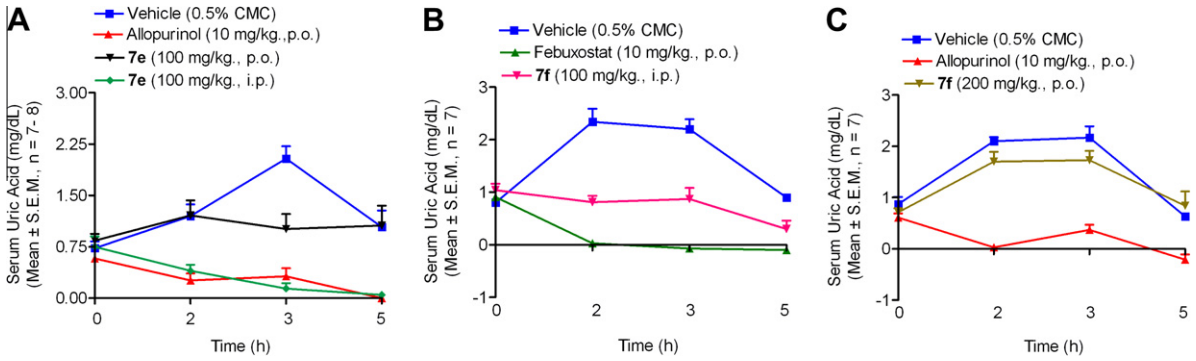


Figure 5. Time course of response of sUA levels for different treatments in hyperuricemic rats. Antihyperuricemic effect of (A) orally and intraperitoneally administered **7e** (B) intraperitoneally administered **7f** and (C) orally administered **7f**.

24 h (Table 2; Fig. 3A shows plot up to 6 h). Due to low oral exposure, efficacy was tested at a high dose of 100 mg/kg po. The reduction in sUA level for **7** in hyperuricemic rat model[8c] was only 23.7% as against 130% for allopurinol (Fig. 4A). This translates to 18% of the efficacy of allopurinol as against a corresponding value of 44% for the original hit, **1**,[8a] in spite of 16-fold improvement in in vitro activity. This is perhaps due to the much poorer oral exposure of **7** (Table 2). PK of another compound, **10**, with better in vitro potency, was studied by both po and ip routes (Fig. 3A). As oral exposure of **10** (Table 2) was worse than that of **7**, its in vivo efficacy was tested by ip route at 100 mg/kg. It showed a reduction of 77% in sUA levels as against 177% and 181% for reference standards, allopurinol and febuxostat, respectively, in the same experiment (Fig. 4B).

R^1 position on **7** was explored next (Table 3, Scheme 1). Functional groups such as $-OCH_3$ (**7d**), $-NO_2$ (**7e**), –CN (**7f**) gave improved IC_{50} values but $-OCF_3$ (**7a**), $-NH_2$ (**7b**) and $-CONH_2$ (**7c**) showed low activity.

Comparison of PK results of the three potent compounds, **7d**, **7e** and **7f**, (Fig 3B, Table 4) shows that ip administration generally gave reasonable C_{max} values. About 5-fold higher C_{max} was obtained for **7d** compared to **10** administered i.p. and it was also tested for efficacy by ip route. The 55% reduction in UA level seen for **7d** (Fig. 4B) was better than that for **7** (23.7%) (Fig. 4A), but not as much as for **10** (77%). This may be due to the fact that **10** is more potent than **7d**. It was therefore felt that further improvement in in vitro potency was required, which was attained by **7e** and **7f**.

7e and **7f** were tested in animal models by both po and ip routes of administration (Fig. 5). **7e** showed reduction of 93% and 133% in sUA levels after po and ip dosing respectively as against allopurinol with 139% reduction (Fig. 5A). Higher enzymatic potency of **7e** seemed to compensate for reduced exposure as compared to **7d**. **7f** also showed reduction of 124% in sUA levels as against febuxostat with 180% reduction (Fig. 5B). However, by po route, it was essentially inactive (Fig. 5C). This may be explained by the PK results (Table 4). A moderate volume of distribution and very high clearance for **7f** resulted in an absolute oral bioavailability (%F) of only 8.6%. Poor oral efficacy could be due to poor absorption, which may be due to both poor dissolution and poor permeability.

In general, greater reduction in sUA levels is achieved by ip than by po route, where the compound bypasses oral absorption and first pass metabolism, indicating that the compounds are active against the molecular target but unable to reach it in vivo.

In summary, a lead compound (IC_{50} = 20 nM) that was 470 times more potent in enzymatic assay than the original hit (IC_{50} = 9.4 μM) was obtained by hit-to-lead efforts. Although it exhibited considerable reduction in sUA levels by ip route as measured by AUC for UA level over 5 h post administration, it did not show oral efficacy equiv to saturation doses of allopurinol and febuxostat. Further efforts at lead optimization to improve PK profile could result in better oral efficacy of this series of compounds.

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