

Supporting Information

Exploration of Novel Xanthine Oxidase Inhibitors Based on 1,6-Dihydropyrimidine-5-Carboxylic Acids by an Integrated in Silico Study

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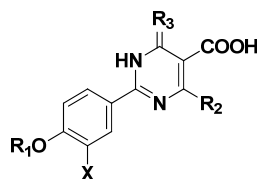
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Table S1. Chemical structures of the used non-purine XOIs with their actual pIC₅₀ values and docking scores.



Compounds 01-26: X = 1H-tetrazol-1-yl
Compounds 27-46: X = CN

No.	R ₁	R ₂	R ₃	IC ₅₀ (μM)	pIC ₅₀	Docking score
01	methyl	H	O	0.0920	7.0362	8.98
02	<i>iso</i> -propyl	H	O	0.0737	7.1325	8.94
03	<i>iso</i> -butyl	H	O	0.0644	7.1911	9.44
04	<i>iso</i> -pentyl	H	O	0.0541	7.2668	9.38
05	allyl	H	O	0.0437	7.3595	9.55
06	<i>iso</i> -butenyl	H	O	0.0569	7.2449	9.46
07	<i>iso</i> -pentenyl	H	O	0.0692	7.1599	9.21
08	propinyl	H	O	0.0500	7.301	9.47
09	methylene cyclopropane	H	O	0.0461	7.3363	9.73
10	cyclopentyl	H	O	0.0585	7.2328	9.51
11	methylene cyclohexane	H	O	0.0683	7.1656	9.46
12	benzyl	H	O	0.0945	7.0246	8.97
13	<i>p</i> -methylbenzyl	H	O	0.0894	7.0487	9.38
14	<i>p</i> -tert-butylbenzyl	H	O	0.1490	6.8268	9.12
15	<i>p</i> -methoxybenzyl	H	O	0.0507	7.295	9.61
16	<i>p</i> -fluorobenzyl	H	O	0.0531	7.2749	9.53
17	<i>p</i> -chlorobenzyl	H	O	0.0691	7.1605	9.35
18	<i>p</i> -bromobenzyl	H	O	0.0552	7.2581	9.46
19	<i>m</i> -methoxybenzyl	H	O	0.0516	7.2874	9.46
20	<i>m</i> -fluorobenzyl	H	O	0.0477	7.3215	9.76
21	<i>m</i> -chlorobenzyl	H	O	0.0288	7.5406	10.63
22	<i>m</i> -bromobenzyl	H	O	0.0450	7.3468	9.67
23	<i>o</i> -chlorobenzyl	H	O	0.0917	7.0376	9.10
24	2,5-dichlorobenzyl	H	O	0.0639	7.1945	9.34
25	2,4-dichlorobenzyl	H	O	0.0838	7.0768	9.37
26	hydrogen	H	O	0.6290	6.2013	7.85
27	<i>iso</i> -propyl	H	O	0.0916	7.0381	9.02
28	<i>iso</i> -butyl	H	O	0.0609	7.2154	9.55
29	<i>iso</i> -pentyl	H	O	0.0250	7.6021	10.51
30	allyl	H	O	0.0811	7.091	9.22
31	<i>iso</i> -butenyl	H	O	0.0336	7.4737	9.91
32	<i>iso</i> -pentenyl	H	O	0.0388	7.4112	9.68
33	benzyl	H	O	0.0387	7.4123	9.78
34	<i>p</i> -fluorobenzyl	H	O	0.0382	7.4179	9.75
35	<i>p</i> -chlorobenzyl	H	O	0.0499	7.3019	9.58
36	<i>p</i> -bromobenzyl	H	O	0.0298	7.5258	10.51
37	<i>p</i> -tert-butylbenzyl	H	O	0.1970	6.7055	8.35
38	<i>p</i> -methylbenzyl	H	O	0.0354	7.451	9.99

39	<i>iso</i> -pentyl	CH ₃	O	0.5400	6.2676	8.40
40	<i>iso</i> -butenyl	CH ₃	O	0.5677	6.2459	8.36
41	<i>p</i> -bromobenzyl	CH ₃	O	0.1854	6.7319	9.01
42	<i>p</i> -methylbenzyl	CH ₃	O	0.1590	6.7986	8.81
43	<i>iso</i> -pentyl	H	NH	0.0240	7.6198	10.68
44	<i>iso</i> -butenyl	H	NH	0.0181	7.7423	10.69
45	<i>p</i> -bromobenzyl	H	NH	0.0271	7.567	10.50
46	<i>p</i> -methylbenzyl	H	NH	0.0339	7.4698	9.97

Table S2. The statistical results of other pharmacophore models using different compounds.

No.	SPECIFICITY	N_HITS	FEATS	PARETO	ENERGY	STERICS	HBOND	MOL_QRY
1	4.811	11	8	0	10.95	2156.90	562.20	95.77
2	4.803	12	8	0	11.80	2163.20	558.40	162.16
3	4.812	12	8	0	10.66	2143.50	562.30	87.70
4	5.638	11	8	0	10.54	2065.30	558.50	80.09
5	4.829	10	8	0	10.12	2191.80	561.10	95.43
6	4.808	12	8	0	8.20	2003.90	526.50	84.06
7	4.825	10	8	0	9.57	1847.80	548.20	76.72
8	4.824	11	8	0	11.54	2059.70	571.10	155.18
9	4.821	12	8	0	10.69	2267.10	584.60	78.31
10	4.822	11	8	0	24.33	2357.60	588.20	80.63

Table S3. The ADME prediction results of the non-ideal virtual-screened hits.

Hit compound	MW (g/mol)	Fraction Csp ³	Rotatable bonds	TPSA (Å ²)	GI absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Lipinski violations	SA score
VS01 (ZINC09434063)	422.61	0.6	13	142.1	Low	No	No	Yes	Yes	Yes	Yes	0	3.43
VS02 (ZINC04705438)	421.52	0.27	7	111	High	No	No	Yes	Yes	No	Yes	0	3.97
VS03 (ZINC59016051)	429.47	0.2	9	126.8	Low	No	No	Yes	Yes	No	Yes	0	3.62
VS04 (ZINC63625083)	427.5	0.23	6	103.1	High	No	No	No	No	No	No	0	4.41
VS05 (ZINC89942644)	318.37	0.38	7	103.2	High	No	No	No	No	No	No	0	3.26
VS06 (ZINC89942901)	370.43	0.44	8	149	Low	No	No	No	No	No	No	0	4.02
VS07 (ZINC04927833)	411.52	0.27	11	101.5	High	No	Yes	Yes	Yes	Yes	Yes	0	3.51
VS08 (ZINC95498920)	347.43	0.63	6	50.8	High	Yes	No	No	No	No	No	0	3.75
VS09 (ZINC39932293)	412.49	0.58	10	176.7	Low	No	No	No	No	No	Yes	1	4.7
VS10 (ZINC12537662)	469.45	0.05	9	151.1	Low	No	No	Yes	No	No	No	1	3.6
VS11 (ZINC12741060)	480.51	0.38	11	123.9	High	No	No	No	Yes	Yes	No	0	4.95
VS13 (ZINC24928355)	295.34	0.33	7	127.2	High	No	Yes	No	No	No	Yes	0	2.76
VS14 (ZINC06053573)	389.36	0.28	9	139.6	High	No	Yes	No	Yes	No	No	0	4.22
VS15 (ZINC31176221)	402.45	0.32	5	96.8	High	No	No	Yes	Yes	No	Yes	0	3.72
VS17 (ZINC21991916)	332.33	0.75	12	151.6	Low	No	No	No	No	No	No	0	3.61
VS18 (ZINC09493835)	463.51	0.47	10	191.3	Low	No	No	Yes	No	No	Yes	0	3.73
VS20 (ZINC65506066)	308.33	0.11	5	57.9	High	Yes	No	No	No	Yes	No	0	2.6
VS21 (ZINC09235475)	426.4	0.09	5	120.3	High	No	No	Yes	No	No	No	0	3.46
VS22 (ZINC32785893)	582.65	0.27	12	123.9	High	No	No	No	No	No	No	0	5.15
VS23 (ZINC21580816)	418.4	0.09	8	122.1	High	No	Yes	Yes	Yes	Yes	Yes	0	2.97
VS24 (ZINC63505952)	498.61	0.33	8	71.5	High	No	No	No	No	Yes	Yes	0	5.42
VS25 (ZINC09295542)	453.81	0.1	7	133.8	Low	No	No	Yes	No	No	No	0	3.47
VS27 (ZINC12820565)	384.44	0.19	6	120.4	High	No	Yes	Yes	Yes	Yes	Yes	0	3.17
VS28 (ZINC16362213)	348.42	0.35	9	106.6	High	No	Yes	Yes	Yes	No	Yes	0	2.87
VS29 (ZINC09120119)	455.55	0.27	8	158.5	Low	No	Yes	Yes	Yes	No	Yes	0	4.11
VS30 (ZINC72320942)	432.56	0.42	8	79.9	High	No	No	Yes	Yes	Yes	Yes	0	4.42

VS31 (ZINC12547951)	387.48	0.26	9	111.1	High	No	Yes	Yes	Yes	No	Yes	0	3.46
VS32 (ZINC39289590)	248.3	0.5	3	38.3	High	Yes	No	No	No	No	No	0	2.68
VS33 (ZINC73707897)	398.48	0.41	10	71.6	High	No	No	No	No	No	Yes	0	4.03
VS34 (ZINC18190685)	481.52	0.3	12	186.9	Low	No	No	No	No	No	Yes	0	3.84
VS35 (ZINC89942526)	369.44	0.37	9	128.2	High	No	No	No	No	No	No	0	3.54
VS36 (ZINC89943165)	370.47	0.5	8	101.4	High	No	No	No	No	No	No	0	3.77
VS37 (ZINC31167456)	502.92	0.43	11	173.7	Low	No	No	No	No	No	No	1	5.28

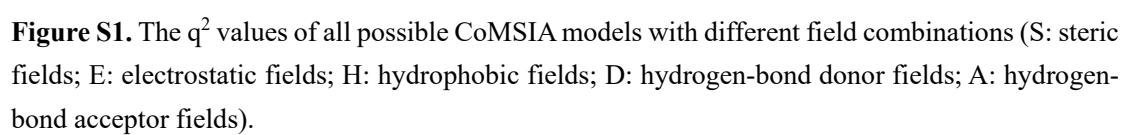


Figure S1. The q^2 values of all possible CoMSIA models with different field combinations (S: steric fields; E: electrostatic fields; H: hydrophobic fields; D: hydrogen-bond donor fields; A: hydrogen-bond acceptor fields).

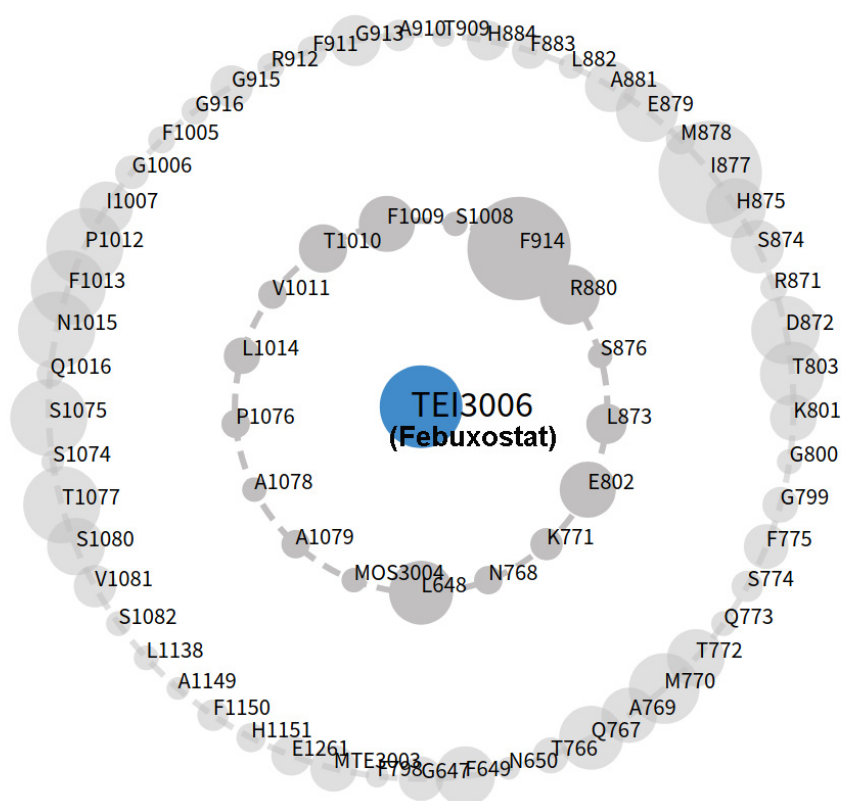


Figure S2. The asteroid plot of co-crystal XO structure (PDB ID: 1N5X) with febuxostat (center node).

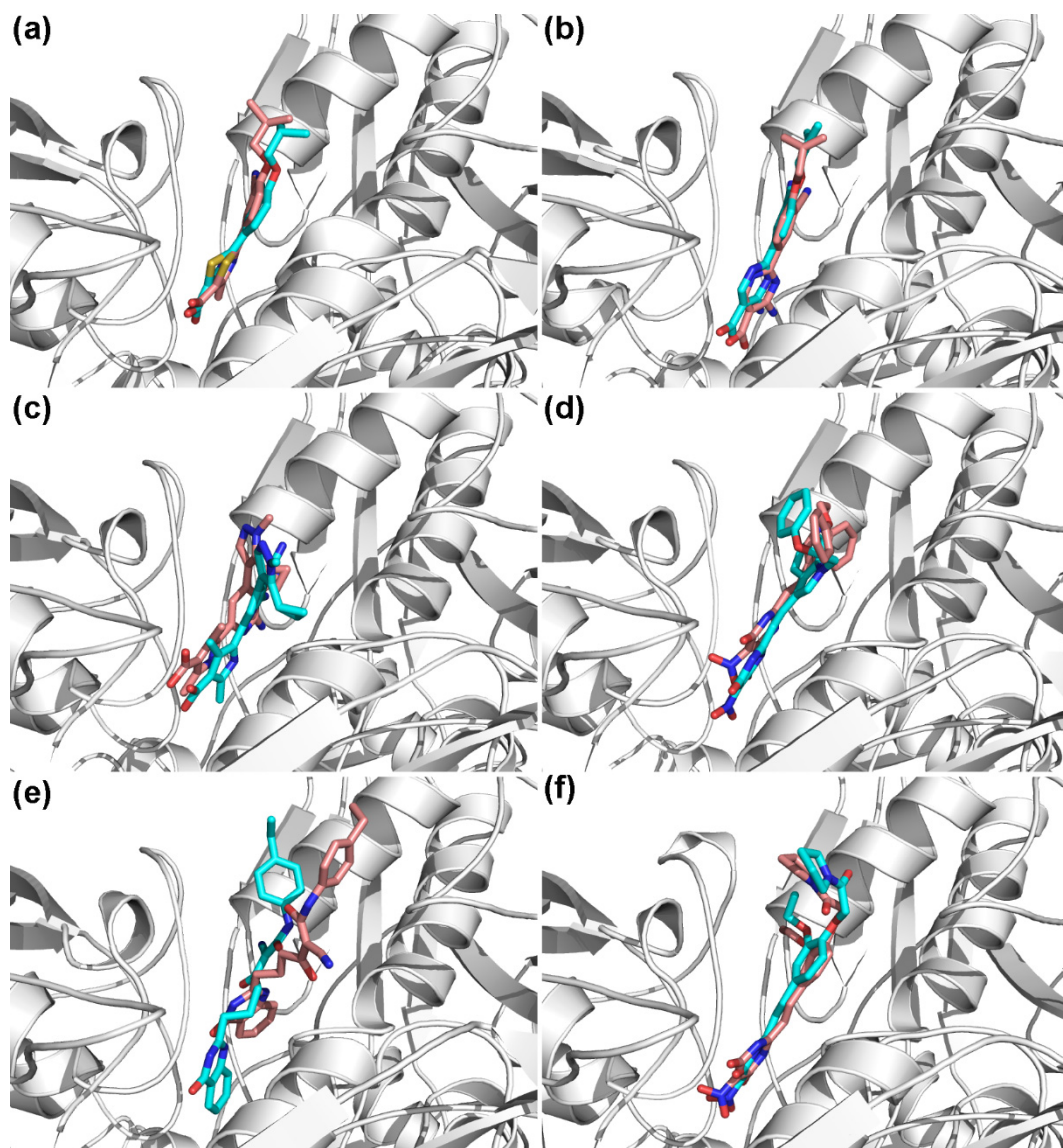


Figure S3. The initial (cyan sticks) and final (pink sticks) conformations of compounds febuxostat (a), 44 (b), VS12 (c), VS16 (d), VS19 (e), and VS26 (f) in XO protein during 50 ns MD simulations.

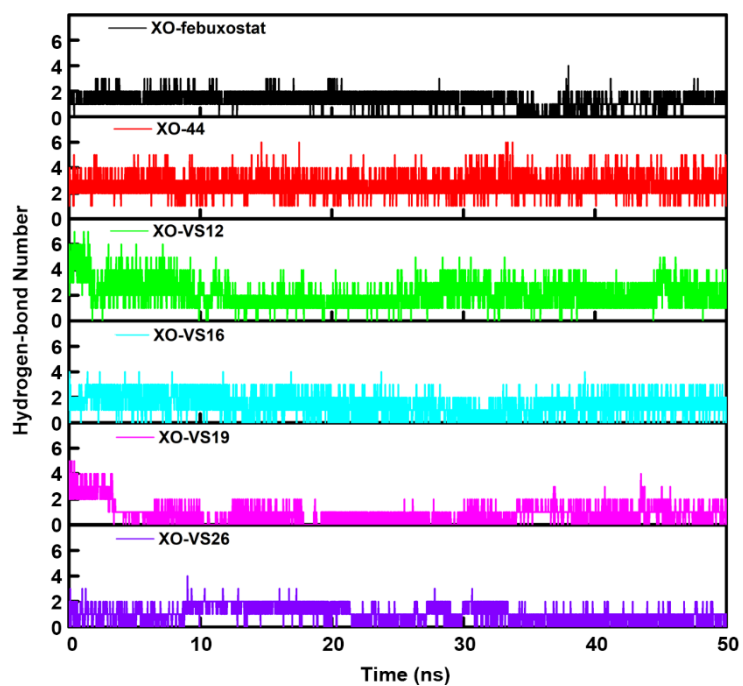


Figure S4. The hydrogen-bond numbers between protein and compounds febuxostat (black), **44** (red), **VS12** (green), **VS16** (cyan), **VS19** (magenta), and **VS26** (violet) during 50 ns MD simulations.

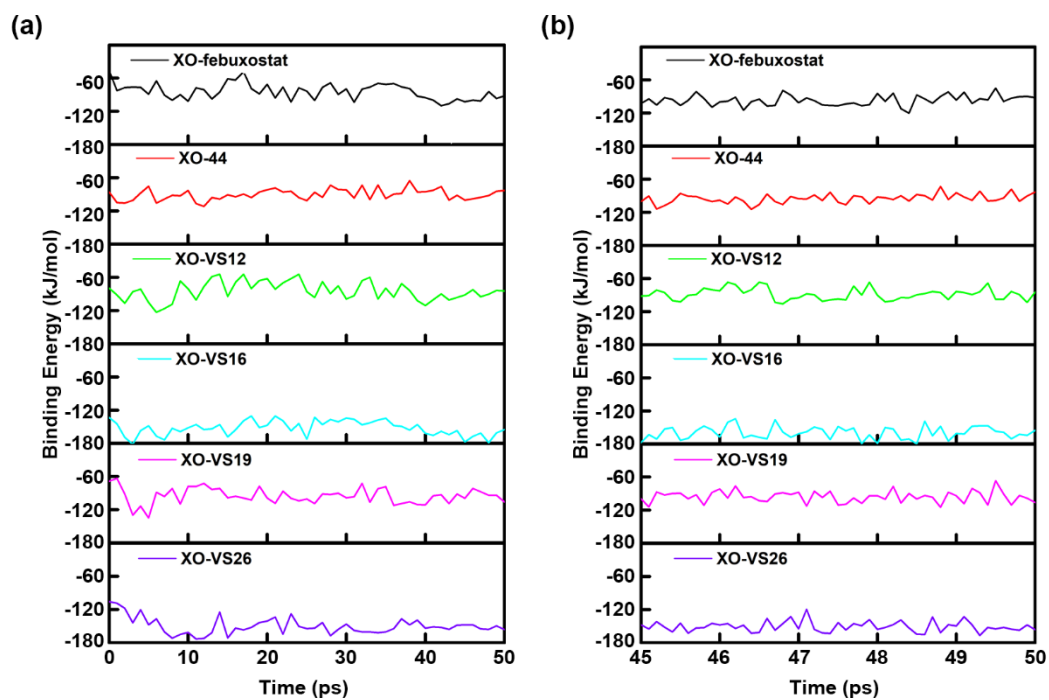


Figure S5. Binding energies (kJ/mol) convergence of complexes XO-febuxostat (black), XO-44 (red), XO-VS12 (green), XO-VS16 (cyan), XO-VS19 (magenta), and XO-VS26 (violet) during 50 ns MD simulations (a: the 50 ns trajectory of each complex at an interval of 1 ns; b: the final 5 ns trajectory of each complex at an interval of 100 ps).

	1	10	20	30	40	50	60
XO_bovine	MTAD	LVFFVNG	KKVVEKNADPETTL	LAYLRRLGL	R	GTKLGC	EGGGCGACTV
XO_HUMAN	MTAD	LVFFVNG	KKVVEKNADPETTL	LAYLRRLGL	S	GTKLGC	EGGGCGACTV
	70	80	90	100	110	120	130
XO_bovine	F	SANACLAP	I	T	LHHVAVTTVEGIGSTK	TRLHPVQ	ERIAKSHGSQ
XO_HUMAN	F	SANACLAP	I	S	LHHVAVTTVEGIGSTK	TRLHPVQ	ERIAKSHGSQ
	140	150	160	170	180	190	200
XO_bovine	T	VEEIED	AFQGNLC	RCTGYRPI	LQGFRTFA	K	NGCCGG
XO_HUMAN	T	VEEIED	AFQGNLC	RCTGYRPI	LQGFRTFA	R	GGCCGG
	210	220	230	240	250	260	
XO_bovine	M	PLDPTQ	EPIFFPELLRL	KD	V	P	KQLRFEG
XO_HUMAN	T	PLDPTQ	EPIFFPELLRL	KD	T	P	RKQLRFEG
	270	280	290	300	310	320	330
XO_bovine	K	FKNQ	LFPMT	ICPAWIPE	LN	A	VEHGP
XO_HUMAN	K	FKNQ	LFPMT	ICPAWIPE	LN	S	VEHGP
	340	350	360	370	380	390	400
XO_bovine	W	FAGKQ	VKS	VASV	GGNIIT	ASPI	SDLNP
XO_HUMAN	W	FAGKQ	VKS	VASV	GGNIIT	ASPI	SDLNP
	410	420	430	440	450	460	
XO_bovine	I	LLSIEI	PYSRE	D	E	F	SFAFKQ
XO_HUMAN	I	LLSIEI	PYSRE	G	E	F	SFAFKQ
	470	480	490	500	510	520	530
XO_bovine	T	QKQLSK	F	W	N	E	K
XO_HUMAN	T	QKQLSK	F	W	K	E	L
	540	550	560	570	580	590	600
XO_bovine	G	KLDPT	T	SATLLF	QKH	P	PAN
XO_HUMAN	G	KLDPT	T	SATLLF	QKH	P	PA
	610	620	630	640	650	660	
XO_bovine	I	F	LRLVT	STRAHAKI	SID	V	SEA
XO_HUMAN	I	S	LRLVT	STRAHAKI	SID	T	SEA
	670	680	690	700	710	720	730
XO_bovine	V	VADTPE	H	A	E	R	A
XO_HUMAN	V	VADTPE	H	T	Q	R	A
	740	750	760	770	780	790	800
XO_bovine	G	GQD	H	FYLETH	CTIA	P	KGE
XO_HUMAN	G	GQD	H	FYLETH	CTIA	P	KGE
	810	820	830	840	850	860	870
XO_bovine	R	STLV	S	A	VALAAY	K	TG
XO_HUMAN	R	STLV	S	A	VALAAY	K	TG
	880	890	900	910	920	930	
XO_bovine	R	DLS	H	SIMER	ALF	H	M
XO_HUMAN	Q	DLS	Q	SIMER	ALF	H	M
	940	950	960	970	980	990	1000
XO_bovine	A	EEV	R	K	N	Y	K
XO_HUMAN	A	EEV	R	K	N	Y	K
	1010	1020	1030	1040	1050	1060	1070
XO_bovine	F	GISFT	V	P	F	L	N
XO_HUMAN	F	GISFT	V	P	F	L	N
	1080	1090	1100	1110	1120	1130	
XO_bovine	P	N	S	P	T	A	S
XO_HUMAN	P	N	T	S	P	T	A

	1140	1150	1160	1170	1180	1190	1200
XO_bovine	GYSFETNSGN ^A FHYF ^T YGVACSEVEIDCLTGDHKNLR ^T DIVMDVGSSLNPAIDIGQVEGAFVQGLGL						
XO_HUMAN	GYSFETNSGN ^P FHYF ^S YGVACSEVEIDCLTGDHKNLR ^T DIVMDVGSSLNPAIDIGQVEGAFVQGLGL						

	1210	1220	1230	1240	1250	1260	1270
XO_bovine	FTLEELHYSPEGSLHTRGPSTYKIPAFGSIP ^T EFRVSLLRDCPNKKAIYASKAVGEPP ^L FL ^G AS ^V VFF						
XO_HUMAN	FTLEELHYSPEGSLHTRGPSTYKIPAFGSIP ^T EFRVSLLRDCPNKKAIYASKAVGEPP ^L FL ^A AS ^I EFF						

	1280	1290	1300	1310	1320	1330
XO_bovine	AIKDAIRAARAQHT ^N NN ^T KELFRLDSPATPEKIRNACVDKFTTLCVTG ^A P ^G NCKPWS ^I RV					
XO_HUMAN	AIKDAIRAARAQHT ^G NN ^V KELFRLDSPATPEKIRNACVDKFTTLCVTG ^V P ^E NCKPWS ^V RV					

Figure S6. The sequence alignment of bovine and human XO enzymes (the key residues for febuxostat binding with XO marked by black frames).

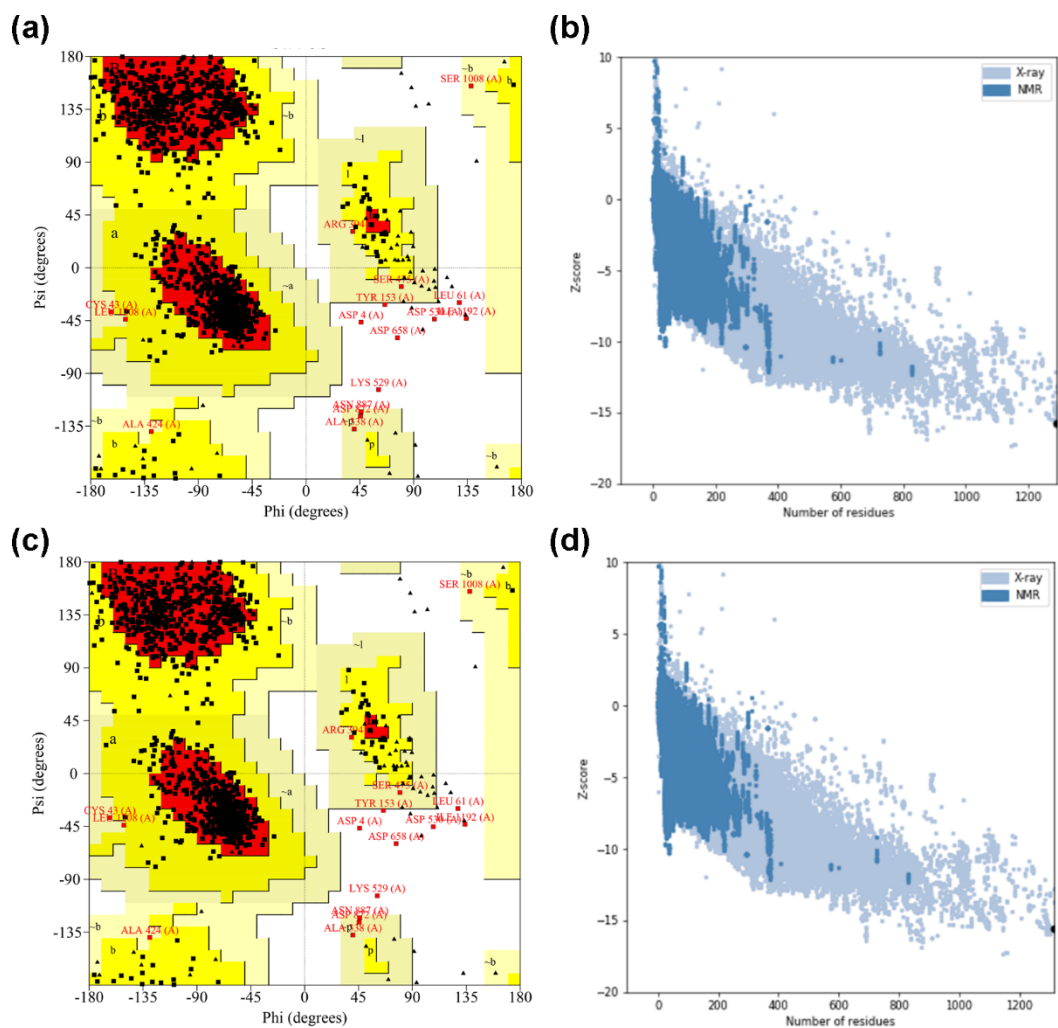


Figure S7. Evaluation results of Ramachandran (a, c) and Z-score distribution (b, d) plots of the original (a, b) and repaired (c, d) XO proteins.