Color polymorphs of aldose reductase inhibitor Epalrestat: configurational, conformational and synthon differences

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Electronic Supplementary Information[†]

 Table S1 Cystallographic parameters of EPR forms.

	Form I	Form I	Form II	Form III	Z,Z isomer
	This study	Reported ^a	This study	This study	This study
Chemical	C15 H13 N	C15 H13 N O3	C15 H13 N	C15 H13 N	C15 H13 N O3
formula	O3 S2	S2	O3 S2	O3 S2	S2
Crystal system	Triclinic	Triclinic	Monoclinic	Monoclinic	Monoclinic
Formula weight	319.38	319.39	319.38	319.38	319.38
Space group	$P\overline{1}$	$P\overline{1}$	<i>C2/c</i>	$P2_1/n$	$P2_1/c$
<i>T</i> [K]	298	298	298	298	298
<i>a</i> [Å]	8.1399(10)	8.20888(15)	13.7384(4)	14.7831(13)	11.4252(5)
<i>b</i> [Å]	11.5584(14)	11.6639(2)	9.1063(3)	5.6288(5)	6.9479(3)
<i>c</i> [Å]	16.1506(19)	16.3107(3)	24.1429(7)	18.2621(16)	19.4673(9)
α[°]	96.721(10)	96.5985(8)	90	90	90
β[°]	93.818(10)	93.8393(8)	100.747(3)	98.364(8)	102.898(5)
γ [°]	104.724(10)	104.6780(8)	90	90	90
Ζ	4	4	8	4	4
<i>V</i> [Å ³]	1452.2(3)	1493.22(5)	2967.44(16)	1503.5(2)	1506.35(12)
$D_{\rm calc} [{ m g cm^{-3}}]$	1.461	1.421	1.430	1.411	1.408

Reflns. collected	9766	16752	10378	6015	5273
Unique reflns.	5131	5319	2625	2654	2663
Observed reflns.	2548	5319	2441	1989	1910
$R_1 [I > 2(I)]$	0.0632	0.0460	0.0558	0.0427	0.0543
$wR_2(all)$	0.1084	0.1315	0.1550	0.0996	0.1603
Goodness-of- fit	0.983	1.081	1.087	1.026	1.036
Diffractometer	Oxford CCD	Rigaku RAXIS- RAPID II	Oxford CCD	Oxford CCD	Oxford CCD

^a Reported crystal structure in ref. 7.

R. Igarashi, H. Nagase, T. Furuishi, T. Endo, K. Tomono and H. Ueda. *X-ray Structure Analysis Online.*, 2013, **29**, 23.

Table S2 Hydrogen b	oond metrics in the	crystal structures.
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Interaction	H···A (Å)	$D \cdots A(Å)$	D–H···A (°)	Symmetry Code		
EPR form I						
O3–H3A…O4	1.98	2.751(6)	174	x,1+y,z		
O6–H6A…O1	1.80	2.664(5)	147	1-x,-y,-z		
C4–H4…O2	2.56	3.419(8)	154	1-x,-y,-z		
С7–Н7…О5	2.40	3.301(6)	164	1-x,-y,-z		
С10-Н10…О5	2.39	3.284(6)	161	1-x,-y,-z		
C24–H24C…S4	2.86	3.796(5)	165	2-x,-y,1-z		
С29–Н29В…О2	2.30	3.212(6)	157	x,-1+y,z		
C19–H19…S2	2.82	3.616(6)	130	x-1,+y,+z		

C21–H21····S3	2.84	3.908(4)	168	x-1,+y,+z		
С9–Н9А…S1	2.39	3.161(6)	137	Intra		
С10-Н10…О1	2.51	2.876(6)	104	Intra		
C14–H14B…O1	2.41	2.773(6)	101	Intra		
C24–H24A…S3	2.54	3.087(5)	116	Intra		
С25-Н25…О4	2.53	2.874(6)	102	Intra		
С29–Н29В…О4	2.47	2.812(5)	100	Intra		
		EPR form	II	I		
O3–H3A…O1	1.97	2.780(3)	168	1/2-x,-1/2+y,1/2-z		
С14-Н14В…О2	2.38	3.330(4)	165	1/2-x,1/2+y,1/2-z		
С10-Н10…82	2.74	3.819(2)	175	x+1/2,+y-1/2,+z		
С4–Н4…О2	2.60	3.389(4)	129	-x+2,-y+2,-z+1		
С9–Н9А…S1	2.56	3.128(3)	118	Intra		
С10-Н10…О1	2.54	2.883(3)	102	Intra		
C14–H14B…O1	2.46	2.839(3)	103	Intra		
	1	EPR form	III			
O3–H3A…O2	1.85	2.670(3)	177	2-x,2-y,1-z		
С10-Н10…О1	2.46	3.345(3)	159	3/2-x,-1/2+y,1/2-z		
С9–Н9А…S1	2.59	3.198(2)	122	Intra		
С10-Н10…О1	2.46	3.345(3)	159	Intra		
C14–H14A…S2	2.74	3.095(3)	102	Intra		
EPR form Z,Z isomer						
O3–H3A…O2	1.84	2.658(3)	177	2-x,1-y,1-z		
С9–Н9С…S1	2.80	3.107(5)	100	Intra		

С10-Н10…О1	2.57	2.903(5)	102	Intra
C14–H14B…O1	2.43	2.815(5)	103	Intra

Table S3 ^{13}C ss-NMR chemical shifts (\delta, ppm) of EPR forms.

Carbon No.	EPR form I	EPR form II	EPR form III	EPR form IV	EPR form V	EPR Z,Z isomer	EPR Amorphous
1	141.7	140.9	143.1	140.2	140.7	143.3	141.4
2	129.8	131.4	129.4	127.6	128.8, 129.8	130.0	128.7
3	134.4, 135.9	134.9	134.8	133.9	133.6, 134.9	134.0	134.9
4	133.3	132.8	132.6	130.8	132.3	134.0	134.9
5	134.4, 135.9	134.9	134.8	133.9	133.6, 134.9	134.0	134.9
6	129.8	131.4	129.4	127.6	128.8, 129.8	130.0	128.7
7	117.4, 120.8	119.7	117.5	117.4	116.4, 119.9, 121.8	122.0	120.6
8	141.7	140.9	143.1	140.2	140.7	143.3	141.4
9	17.8, 14.9	16.3	17.0	14.5	14.0, 16.9, 19.8	20.0	15.4
10	145.2, 147.9	145.2	146.8	145.6	144.2, 146.9	143.3	141.4
11	127.4	130.9	127.6	125.9	126.5	128.9	128.7
12	191.8, 193.9	191.0	192.6	191.5	190.9, 193.0	193.6	192.2
13	167.8	167.6	166.3	163.7	166.8	165.7	166.4
14	43.0	43.0	43.7	42.8	42.2, 44.4	44.6	43.9
15	167.8	167.6	173.7	175.1	174.0	174.3	173.4

Drug Forms	T _{trs/fus} (°C) T _{onset} / T _{peak}	ΔH _{fus} kJ/mol	ΔH _{trs} kJ/mol	Stability relation
Form I	221.94/224.40	42.96	-	-
Form II	215.83/218.11	43.34	+0.38, (II→I)	enantiotropic
Form III	196.53/199.60	42.29	-0.67 , (III \rightarrow I)	monotropic
Form IV	166.09/172.42	46.75	-2.17, (IV→V)	monotropic
Form V	200.18/207.08	48.92	+5.96, (V→1)	enantiotropic
Amorphous	82.20/88.92	-	-	-
Z,Z isomer	178.97/190.82	-	-	-

Table S4 Transition temperature and enthalpy values of EPR forms.^a

^a T_{fus} = melting point; T_{trs} = transition temperature; ΔH_{fus} = enthalpy of fusion; ΔH_{trs} = enthalpy of transition.

Enthalpy of fusion (ΔH_{fus}) of form I was determined directly from DSC, since it doesn't show any phase transformation.

 ΔH_{fus} values of remaining forms were calculated as follows.

 $\Delta H_{fus (form 2)} = \Delta H_{fus (form 1)} + \Delta H_{trs}$

 $\Delta H_{fus \text{ (form 3)}} = \Delta H_{fus \text{ (form 1)}} + \Delta H_{trs}$

 $\Delta H_{fus \text{ (form 5)}} = \Delta H_{fus \text{ (form 1)}} + \Delta H_{trs}$

 $\Delta H_{fus \text{ (form 4)}} = \Delta H_{fus \text{ (form 5)}} + \Delta H_{trs}$

Phase transitions from II to I and V to I are endothermic (DSC), while transitions from III to I and IV to V are exothermic and there is no direct phase transformation from form IV to I.

Accordingly form I is enantiotropically related to forms II, V, and monotropically related to forms III, IV; Form IV and V also monotropically related.

Based on T_{onset} in DSC (°C), the stability of polymorphs may be ordered as Form I (221.94, most stable) > Form II (215.83) > Form V (200.18) > Form III (196.53) > Form IV (166.09, least stable).

Polymorph	1 M	2 M	3 M	4 M	5 M	6 M
EPR form I	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
EPR form II	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	
EPR form III		\checkmark	\checkmark	V	\checkmark	\checkmark
EPR form IV	V	\checkmark	\checkmark	V	V	\checkmark
EPR form V	V	\checkmark	\checkmark	V	\checkmark	\checkmark

Table S5 Stability of Epalrestat Polymorphs under ICH Conditions of 40 °C and 75% RH.^a

M = month, $\sqrt{1}$ = no phase change/no hydrate formation

^a see Figure S17 to S22 for PXRD plots on stability study



Figure S1 An overlay diagram of the conformers found in the EPR forms. Color codes: Redmolecule A of EPR form I, Green-molecule B of EPR form I, Yellow-EPR form II, Blue-EPR form III, Cyan EPR Z,Z isomer.



Figure S2 Tetrameric motif EPR molecules were extend in to a chain through C–H \cdots S interactions in the crystal structure of form I



Figure S3 Overlay of experimental PXRD (black) of EPR forms I, II, III and Z,Z isomer match with the calculated lines from the crystal structure (red).



Figure S4 Experimental PXRD pattern of EPR forms.



Figure S5 FT-IR spectral overlay of EPR forms.



Figure S6 FT-Raman spectral overlay of EPR forms.



Figure S7 DSC thermogram of EPR amorphous form showing phase transformations.



Figure S8 DSC thermogram of EPR Z,Z isomer.



Figure S9 VT-PXRD of EPR amorphous form (green) shows transformation to form IV upon heating.



Figure S10 VT-PXRD of EPR form IV (red) shows transformation from form IV to form V started at 160 °C (green) and completed at 180 ° (purple) and form V is stable upon cooling.



Figure S11 VT-PXRD of EPR form V (pink) shows transformation from form V to form I started at 190 °C (green) and completed at 200 °C (red) and form I is stable upon cooling.



Figure S12 VT-PXRD of EPR form II (blue) shows transformation from form II to form I started at 190 °C (dark blue) and completed at 200 °C (red) and form I is stable upon cooling.



Figure S13 VT-PXRD of EPR form III (blue) shows transformation from form III to form I started at 190 °C (cyan) and completed at 200 °C (red) and form I is stable upon cooling.



Figure S14 VT-PXRD of EPR Z,Z isomer (brown) shows transformation to form I of E,Z isomer upon heating at 190 °C (green) and form I is stable upon cooling.

30 °C	220 °C	226 °C	230 °C

(a)

30 °C (orange)	183 °C	190°C (dark red)	238 °C			

(b)

0	-		
30 °C (yellow)	168 °C	170 °C (red)	228 °C

(c)



Figure S15 HSM snapshots of EPR polymorphs. a) No changes were observed in crystal morphology of form I. b) Orange color crystals of form II darkened between 183-190°C indicating phase transition. c) Yellow color of form III crystals turned to red between 168-170°C

indicating phase transition. d) Yellow color of Z,Z isomer crystals turned to red between 177-182°C indicating its conversion to form I of E,Z isomer.



Figure S16 Possible reaction mechanism and qualitative energy diagram to show the transformation of E,Z-EPR to ZZ-EPR and then EZ-EPR polymorph.



Figure S17 Stability of EPR amorphous form at ambient conditions shows that after 3 days it is transforming to form IV.



Figure S18 Stability study at 40 °C and 75% RH of EPR form I. There is no change in the physical form after 6 months.



Figure S19 Stability study at 40 °C and 75% RH of EPR form II. There is no form change up to 6 months.



Figure S20 Stability study at 40 °C and 75% RH of EPR form III shows that form III is stable up to 6 months.



Figure S21 Stability study at 40 °C and 75% RH of EPR form IV. There is no form change up to 6 months.



Figure S22 Stability study at 40 °C and 75% RH of EPR form V. There is no form change up to 6 months.



Figure S24 ¹H NMR spectrum of EPR form II (DMSO-d₆).



Figure S26 ¹H NMR spectrum of EPR form IV (DMSO-d₆).



Figure S28 ¹H NMR spectrum of EPR Z,Z isomer (DMSO-d₆).



Figure S29 ¹H NMR spectral overlay of EPR forms recorded in d_6 -DMSO. Inter-conversion of the E,Z and Z,Z isomers in solution is seen based on the separation of proton signals at 4.7-4.8 (2H, N-CH₂) and the peak at 7.6-7.7 (1H, most downfield aromatic). This olefin isomerization was reported earlier by T. Ishida, Y. In, M. Inoue, Y. Ueno and C. Tanaka. *Tetrahedron Lett.*, 1989, **30**, 959. The singlet at 3.35 is DMSO proton signal.

Experimental Section

Materials and Methods

Epalrestat was gift sample from Symed Laboratories Ltd., Hyderabad, India and used without further purification. All other chemicals were of analytical or chromatographic grade. Water purified from a deionizer-cum-mixed-bed purification system (AquaDM, Bhanu, Hyderabad, India) was used in the experiments.

Preparation of Epalrestat Forms

EPR form I

The commercial material obtained from Symed Laboratories matches to Form-1 which was confirmed by PXRD and DSC. Block shaped red color single crystals suitable for X-ray diffraction were obtained after 3-4 d upon dissolving 30 mg of EPR in 10 mL of hot ethyl acetate : nitromethane solvent mixture (1:1) and left for slow evaporation at ambient conditions. Figure S23 shows the ¹H NMR spectrum of EPR form I.

¹H NMR (DMSO-d₆, 500 MHz) δ ppm: 13.46 (1 H, br s), 7.63 (1 H, s), 7.49-7.40 (4 H, m), 7.40-7.35 (2 H, m), 4.73 (2H, s), 2.23 (3 H, s).

EPR form II

Diamond shaped orange color single crystals suitable for X-ray diffraction were obtained after 3-4 d upon dissolving 30 mg of EPR in 5 mL of hot 2-propanol solvent and left for slow evaporation at ambient conditions. Form II was obtained in bulk upon dissolving 30 mg of EPR in 10 mL of methanol and heated to get clear solution. This hot solution was rotavaporized immediately to get solid. Formation of form II was confirmed by FT-IR, FT-Raman, ss-NMR, PXRD and DSC. Figure S24 shows the ¹H NMR spectrum of EPR form II.

¹H NMR (DMSO-d₆, 500 MHz) δ ppm: 13.46 (1 H, br s), 7.63 (1 H, s), 7.49-7.40 (4 H, m), 7.40-7.35 (2 H, m), 4.73 (2H, s), 2.23 (3 H, s).

EPR form III

Needle morphology yellow color single crystals suitable for X-ray diffraction were obtained after 3-4 d upon dissolving 30 mg of EPR in 10 mL of hot methanol: benzene solvent mixture (1:1) and left for slow evaporation at ambient conitions. Form III was obtained in bulk upon dissolving 50 mg of EPR in 20 mL of nitromethane and heated to get clear solution. This hot solution cooled immediately by keeping in ice to get precipitate. The precipitate was filtered and the formation of form III was confirmed by FT-IR, FT-Raman, ss-NMR, PXRD and DSC. Figure S25 shows the ¹H NMR spectrum of EPR form III.

¹H NMR (DMSO-d₆, 500 MHz) δ ppm: 13.45 (1 H, s), 7.63 (1 H, s), 7.49-7.40 (4 H, m), 7.40-7.35 (2 H, m), 4.73 (2H, s), 2.23 (3 H, s).

EPR form IV

EPR form IV was obtained upon heating of amorphous form at 85 °C for 30 min in programmable oven. The formation of form IV was confirmed by FT-IR, FT-Raman, ¹³C ss-NMR, PXRD, and DSC. Attempts to crystallize the form IV in ethanol, CH₃CN, THF, nitromethane, toluene, acetone, and also mixture of solvents gave polycrystalline powders but no diffraction quality single crystals were obtained. Figure S26 shows the ¹H NMR spectrum of EPR form IV.

¹H NMR (DMSO-d₆, 500 MHz) δ ppm: 13.45 (1 H, s), 7.64 (1 H, s), 7.50-7.40 (4 H, m), 7.40-7.30 (2 H, m), 4.73 (2H, s), 2.23 (3 H, s).

EPR form V

EPR form IV was obtained upon heating of amorphous form at 160 °C for 30 min in programmable oven. The formation of form IV was confirmed by FT-IR, FT-Raman, ¹³C ss-NMR, PXRD, and DSC. Attempts to crystallize the form V in ethanol, CH₃CN, THF, nitromethane, toluene, acetone, and also mixture of solvents gave polycrystalline powders but no diffraction quality single crystals were obtained. Figure S27 shows the ¹H NMR spectrum of EPR form V.

¹H NMR (DMSO-d₆, 500MHz) δ ppm: 13.46 (1 H, s), 7.63 (1 H, s), 7.49-7.40 (4 H, m), 7.40-7.20 (2 H, m), 4.60 (2H, s), 2.20 (3 H, s).

EPR amorphous form

100 mg of EPR was melted at 225 °C and then immediately dipped in liquid nitrogen to cool down to a glassy state. Formation of amorphous form was confirmed by FT-IR, FT-Raman, ¹³C ss-NMR, PXRD, and DSC.

EPR Z,Z isomer

Plate morphology yellow color single crystals suitable for X-ray diffraction were obtained after 3-4 d upon dissolving 30 mg of EPR in 10 mL of various solvent mixtures (1:1) such as tetrahydrofuran : heptane, ethanol : benzene, methanol : 1,2-dibromoethane and left for slow evaporation at ambient conditions. Figure S28 shows the ¹H NMR spectrum of EPR Z,Z isomer.

¹H NMR (DMSO-d₆, 500 MHz) δ ppm: 13.48 (1 H, s), 7.66 (1 H, s), 7.50-7.35 (2 H, m), 7.40-7.35 (1 H, t), 7.33-7.30 (2 H, d), 7.23 (1 H, s), 4.68 (2H, s), 2.24 (3 H, s).

Polymorphic Stability under ICH Conditions

EPR polymorphs were found to be stable in ambient conditions of Hyderabad (35 °C and 40% RH) for more than one year. Polymorphs will undergo phase transformations or hydrate formation on exposure to humidity. Therefore EPR polymorphs were tested for form stability in accelerated conditions of 40 °C and 75% RH. EPR polymorphs I to V did not exhibit any polymorphic change or hydrate formation in the test period of six months (Figure S18 to S22).

Grinding and Slurry experiments

Grinding experiments were performed at 100 mg scale. A Retsch mixer-mill equipped with a 5 mL stainless steel grinding jar and SS balls of 4 mm diameter was used for mechanical grinding. EPR forms I, II, and V did not exhibit any phase transformation even after 2 h of solid-state grinding, whereas form IV was converted to form I after 20 min and form III was transformed to form I in 30 min. Slurry experiments were also performed at 100 mg scale. Water (5 mL in each experiment) was used as solvent in slurry experiments. The slurry was stirred at 800 rpm using a magnetic stirrer at 30 °C. After 5 d, the suspension was filtered through Whatman's filter paper No. 1 and the dried material was characterized by PXRD. No phase transformations of form I, II, IV and V were observed even after 5 d, but form III converted to form I after 24 h. Based on the grinding and slurry experiments as well as thermal analysis, EPR polymorphs are ranked in the following stability order: Form I (thermodynamic) > Form II > Form V > Form III > Form IV (least stable).

Table S6 Stability of Epalrestat Polymorphs under mechanical grinding and slurry experiments.

Solid form	mechanical hours)	grinding	(dry	grinding,	2	Slurry grinding (in water, 5 days)
Form I	Stable					Stable
Form II	Stable					Stable

Form III	Transformed to form I (30 min)	Transformed to form I (after 24 hrs)
Form IV	Transformed to form I (20 min)	Stable
Form V	Stable	Stable
