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New Synthetic Isoxazole Derivatives acting as potent Inducers of Fetal Hemoglobin in Erythroid Precursor Cells isolated from β-Thalassemic Patients

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Supplementary material

HU

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	108.0	8.9	1.0	115.0	14.3	1.0	93.5	2.5	1.0	79.2	4.0	1.0	109.2	15.4	1.0	43.4	3.2	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.3	559.0	44.8	4.9	1054.7	74.4	11.3	331.0	40.6	4.2	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	114.0	5.2	1.1	93.0	6.9	0.8	88.0	3.8	0.9	118.8	28.6	1.5	88.2	12.8	0.8	97.1	9.8	1.0	84.0	2.6	1.4	89.8	5.1	1.5
5	116.0	20.0	1.1	80.5	11.4	0.7	78.1	3.8	0.8	106.7	13.3	1.3	90.3	11.1	0.8	109.7	21.2	1.1	76.7	6.8	1.3	**114.7**	**13.3**	**2.0**
10	59.0	6.9	0.5	68.0	0.9	0.6	93.5	7.6	1.0	89.1	5.7	1.1	99.4	17.0	0.9	87.7	5.4	0.9	89.5	10.0	1.5	109.3	2.3	1.9
50	98.0	49.5	0.9	98.0	7.5	0.9	95.7	15.1	1.0	123.2	10.1	1.6	122.5	11.6	1.1	134.7	11.8	1.4	73.7	4.6	1.3	73.5	14.5	1.3
100	96.0	3.0	0.9	62.0	3.8	0.5	78.1	6.9	0.8	127.6	6.9	1.6	116.9	2.4	1.1	153.5	33.3	1.6	59.0	11.3	1.0	54.5	8.3	0.9

Table S1. Lack of genotoxic activity of Hydroxyurea (HU) at different concentrations (1, 5, 10, 50, 100 μg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 μg/plate).

C1

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	108.0	8.9	1.0	138.0	9.5	1.0	93.5	8.3	1.0	79.2	13.2	1.0	109.2	15.4	1.0	97.1	3.2	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.3	559.0	44.8	4.0	1054.7	74.4	10.0	331.0	40.6	4.2	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	68.1	27.2	0.6	151.2	14.7	1.1	67.1	1.9	0.7	83.6	5.0	1.1	132.3	4.2	1.2	126.9	16.3	1.3	42.5	0.0	0.7	52.7	7.2	0.9
5	84.6	9.0	0.8	165.0	22.4	1.2	53.9	1.9	0.6	82.5	0.0	1.0	135.1	2.4	1.2	111.2	10.9	1.1	30.8	3.2	0.5	74.0	1.0	1.3
10	83.7	17.6	0.8	157.8	3.7	1.1	70.4	3.8	0.8	83.6	1.9	1.1	146.3	17.1	1.3	125.3	15.1	1.3	31.7	1.4	0.5	82.3	2.1	1.4
50	76.3	16.5	0.7	145.8	4.8	1.1	62.7	11.4	0.7	67.1	19.3	0.8	122.5	5.3	1.1	103.4	18.8	1.1	50.3	2.0	0.9	83.3	9.8	1.4
100	66.4	6.0	0.6	132.6	15.5	1.0	53.9	15.6	0.6	63.8	3.8	0.8	123.2	17.5	1.1	139.4	5.4	1.4	39.8	1.0	0.7	70.8	1.0	1.2

Table S2. Lack of genotoxic activity of **c1** at different concentrations (1, 5, 10, 50, 100 μg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 μg/plate).

C2

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102.0	6.0	1.0	109.0	6.9	1.0	105.2	4.0	1.0	137.4	4.7	1.0	109.2	15.4	1.0	97.1	15.1	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.4	559.0	44.8	5.1	1054.7	74.4	10.0	331.0	40.6	2.4	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	90.2	5.2	0.9	112.1	32.9	1.0	143.5	16.4	1.4	125.0	1.8	0.9	115.5	3.6	1.1	87.7	7.2	0.9	31.0	4.1	0.5	51.7	2.9	0.9
5	62.0	4.6	0.6	99.0	37.5	0.9	97.0	43.5	0.9	146.7	10.9	1.1	153.3	15.9	1.4	87.7	17.8	0.9	37.8	0.6	0.6	61.7	9.5	1.1
10	90.3	9.0	0.9	125.5	14.2	1.1	143.5	0.0	1.4	130.2	6.2	0.9	134.4	23.4	1.2	78.3	26.7	0.8	37.2	0.3	0.6	63.0	0.0	1.1
50	70.1	4.6	0.7	149.4	20.0	1.4	139.4	14.2	1.3	138.5	22.4	1.0	141.4	2.4	1.3	72.1	16.5	0.7	39.5	3.0	0.7	76.3	5.5	1.3
100	66.0	13.7	0.6	141.0	7.9	1.3	**214.6**	**22.6**	**2.0**	106.4	3.6	0.8	144.9	17.2	1.3	70.5	12.4	0.7	21.7	4.0	0.4	57.0	6.6	1.0

Table S3. Lack of genotoxic activity of **c2** at different concentrations (1, 5, 10, 50, 100 μg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 μg/plate).

C3

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102.0	6.0	1.0	109.0	6.9	1.0	105.2	4.0	1.0	137.4	4.7	1.0	109.2	15.4	1.0	97.1	15.1	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.4	559.0	44.8	5.1	1054.7	74.4	10.0	331.0	40.6	2.4	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	190.0	17.1	1.8	85.4	18.3	0.8	121.6	17.1	1.2	150.9	7.8	1.1	85.4	2.4	0.8	84.6	12.4	0.9	53.7	1.2	0.9	40.7	1.5	0.7
5	197.2	22.7	1.9	88.0	13.9	0.8	108.0	12.5	1.0	132.3	7.8	1.0	137.9	9.5	1.3	81.5	9.8	0.9	41.7	1.4	0.7	66.7	9.2	1.1
10	173.6	13.9	1.7	102.6	15.9	0.9	110.7	18.8	1.1	139.5	37.6	1.0	135.1	2.4	1.2	90.9	28.7	1.0	37.3	4.6	0.6	51.0	5.3	0.9
50	170.6	17.6	1.7	104.2	6.9	0.9	132.6	10.3	1.3	124.0	3.1	0.9	138.6	5.6	1.3	103.4	12.4	1.1	29.5	2.3	0.5	57.3	0.6	1.0
100	166.5	32.9	1.6	82.2	1.7	0.8	102.5	8.2	1.0	132.3	1.8	1.0	137.2	1.2	1.3	70.5	12.4	0.7	49.8	0.6	0.8	52.0	5.6	0.9

Table S4. Lack of genotoxic activity of **c3** at different concentrations (1, 5, 10, 50, 100 µg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 µg/plate).

C4

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	108.0	8.9	1.0	138.0	9.5	1.0	93.5	8.3	1.0	79.2	13.2	1.0	109.2	15.4	1.0	97.1	3.2	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.3	559.0	44.8	4.0	1054.7	74.4	11.3	331.0	40.6	4.2	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	100.3	20.4	0.9	129.0	14.5	0.9	63.8	3.8	0.7	73.7	3.8	0.9	97.3	2.4	0.9	120.6	19.6	1.2	49.5	5.5	0.8	75.8	14.1	1.3
5	108.7	18.0	1.0	144.0	8.2	1.0	57.2	15.2	0.6	68.2	21.0	0.9	91.0	1.2	0.8	119.1	17.8	1.2	70.8	14.9	1.2	41.2	4.3	0.7
10	97.0	14.2	0.9	148.2	5.8	1.1	50.6	24.8	0.5	135.3	34.9	1.7	104.3	17.6	1.0	139.4	2.7	1.4	64.0	10.5	1.1	48.0	5.2	0.8
50	82.6	22.5	0.8	151.2	26.5	1.1	45.1	3.8	0.5	115.5	5.7	1.5	109.9	11.6	1.0	158.2	11.8	1.6	73.5	0.5	1.2	51.0	8.2	0.9
100	83.1	20.4	0.8	138.6	12.5	1.0	40.7	10.6	0.4	119.9	3.8	1.5	105.0	8.4	1.0	115.9	17.8	1.2	77.2	13.1	1.3	48.2	8.0	0.8

Table S5. Lack of genotoxic activity of **c4** at different concentrations (1, 5, 10, 50, 100 µg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 µg/plate).

C5

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102.0	6.0	1.0	109.0	6.9	1.0	105.2	4.0	1.0	137.4	4.7	1.0	109.2	15.4	1.0	97.1	15.1	1.0	58.8	4.8	1.0	58.0	11.8	1.0
C+	351.0	35.8	3.4	559.0	44.8	5.1	1054.7	74.4	10.0	331.0	40.6	2.4	478.4	26.9	4.4	285.0	5.4	2.9	220.1	3.0	3.9	191.7	5.3	3.3
1	109.1	36.7	1.1	155.1	3.5	1.4	132.6	8.5	1.3	126.1	10.9	0.9	135.8	3.2	1.2	73.6	5.4	0.8	47.3	1.8	0.8	54.2	1.6	0.9
5	87.9	6.9	0.9	151.2	11.4	1.4	129.8	9.5	1.2	139.5	16.1	1.0	122.5	3.2	1.1	109.7	17.8	1.1	47.3	2.3	0.8	62.7	5.9	1.1
10	86.5	1.7	0.8	177.4	20.8	1.6	121.6	9.5	1.2	107.5	4.7	0.8	123.9	7.3	1.1	98.7	9.4	1.0	51.3	6.8	0.9	55.5	2.6	1.0
50	76.0	1.7	0.7	133.0	7.5	1.2	120.3	22.6	1.1	151.9	0.0	1.1	126.7	15.8	1.2	70.5	0.0	0.7	64.5	11.8	1.1	50.3	1.4	0.9
100	92.4	1.7	0.9	90.3	6.0	0.8	131.2	7.1	1.2	156.0	14.3	1.1	113.4	5.6	1.0	78.3	2.7	0.8	38.2	0.6	0.6	57.7	3.0	1.0

Table S6. Lack of genotoxic activity of **c5** at different concentrations (1, 5, 10, 50, 100 μg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 μg/plate).

C6

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102.0	6.6	1.0	144.5	17.8	1.0	90.3	7.6	1.0	82.0	7.0	1.0	112.7	6.0	1.0	136.9	14.8	1.0	55.8	6.5	1.0	65.8	10.4	1.0
C+	351.0	35.8	3.4	559.0	44.8	3.9	1054.7	74.4	11.7	331.0	40.6	4.0	478.4	26.9	4.2	285.0	5.4	2.1	220.1	3.0	3.9	191.7	5.3	2.9
1	74.0	18.3	0.7	165.0	5.4	1.2	65.8	16.8	0.7	86.7	3.1	1.1	127.4	6.1	1.1	107.3	6.4	0.8	67.3	0.6	1.2	77.2	16.3	1.2
5	133.1	3.1	1.3	192.2	11.3	1.3	83.3	8.7	0.9	81.0	11.1	1.0	115.5	3.6	1.0	148.0	6.4	1.1	65.5	3.5	1.2	73.0	2.0	1.1
10	179.3	9.0	1.8	157.1	25.7	1.2	75.6	12.8	0.8	75.0	2.0	0.9	138.6	3.6	1.2	169.0	28.0	1.2	85.0	14.7	1.5	73.8	6.8	1.1
50	152.8	14.0	1.5	128.0	16.5	0.9	73.5	16.4	0.8	66.3	0.6	0.8	144.9	7.6	1.3	231.9	20.4	1.7	65.2	2.0	1.2	67.7	1.2	1.0
100	99.3	14.7	1.0	90.3	8.4	0.6	100.1	4.4	1.1	19.7	4.9	0.2	146.3	4.8	1.3	169.0	29.9	1.2	70.3	9.1	1.3	78.3	4.6	1.2

Table S7. Lack of genotoxic activity of **c6** at different concentrations (1, 5, 10, 50, 100 μg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 μg/plate).

C7

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102,0	6,6	1,0	144,5	17,8	1,0	90.3	7.6	1.0	82.0	7.0	1.0	112.7	6.0	1.0	136.9	14.8	1.0	55.8	6.5	1.0	65.8	10.4	1.0
C+	351.0	35.8	3.4	559.0	44.8	3.9	1054.7	74.4	11.7	331.0	40.6	4.0	478.4	26.9	4.2	285.0	5.4	2.1	220.1	3.0	3.9	191.7	5.3	2.9
1	87,3	4,6	0,9	130,5	10,5	0,9	81.2	5.3	0.9	70.7	5.8	0.9	191.1	12.8	1.7	141.8	20.4	1.0	76.8	8.6	1.4	83.7	17.2	1.3
5	93,3	3,1	0,9	118,0	13,9	0,8	68.6	12.3	0.8	84.3	8.5	1.0	174.3	15.1	1.5	99.9	6.4	0.7	76.3	6.0	1.4	95.0	10.8	1.4
10	102,7	4,2	1,0	112,0	10,2	0,8	70.0	15.8	0.8	84.7	17.9	1.0	179.2	20.7	1.6	156.6	7.7	1.1	72.8	7.8	1.3	74.0	13.0	1.1
50	131,3	8,1	1,3	76,5	1,5	0,5	77.7	4.2	0.9	65.3	5.0	0.8	157.5	0.0	1.4	144.3	0.0	1.1	74.8	1.2	1.3	87.7	2.5	1.3
100	108,0	5,3	1,1	78,5	17,9	0,5	78.4	1.2	0.9	103.3	16.0	1.3	158.9	6.1	1.4	169.0	59.8	1.2	71.2	5.6	1.3	83.2	1.6	1.3

Table S8. Lack of genotoxic activity of **c7** at different concentrations (1, 5, 10, 50, 100 µg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 µg/plate).

C8

	Salmonella typhimurium **TA97A**						*Salmonella typhimurium* **TA98**						*Salmonella typhimurium* **TA100**						*Salmonella typhimurium* **1535**					
	- S9			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**			**- S9**			**+ S9**		
	average	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**	**average**	**SD**	**t/c**
DMSO	102.0	6.6	1.0	136.7	15.0	1.0	90.3	7.6	1.0	82.0	7.0	1.0	112.7	6.0	1.0	136.9	14.8	1.0	55.8	6.5	1.0	65.8	10.4	1.0
C+	351.0	35.8	3.4	559.0	44.8	4.1	1054.7	74.4	11.7	331.0	40.6	4.0	478.4	26.9	4.2	285.0	5.4	2.1	220.1	3.0	3.9	191.7	5.3	2.9
1	84.7	5.8	0.8	168.2	39.3	1.2	58.8	5.6	0.7	73.3	2.9	0.9	98.7	9.2	0.9	119.6	22.3	0.9	60.3	9.5	1.1	71.8	15.1	1.1
5	77.3	1.2	0.8	141.2	11.1	1.0	68.6	16.0	0.8	72.0	0.0	0.9	101.5	3.2	0.9	129.5	3.7	0.9	61.7	1.2	1.1	60.7	4.5	0.9
10	71.2	4.6	0.7	127.7	6.7	0.9	156.8	1.2	1.7	44.3	4.0	0.5	98.7	18.7	0.9	161.6	11.9	1.2	72.3	1.2	1.3	72.2	11.6	1.1
50	72.0	3.5	0.7	127.3	19.2	0.9	107.1	7.3	1.2	83.3	19.4	1.0	106.4	10.4	0.9	152.9	18.3	1.1	47.3	0.6	0.8	61.7	10.4	0.9
100	103.7	7.0	1.0	124.3	5.5	0.9	81.9	4.2	0.9	86.7	9.3	1.1	140.7	2.1	1.2	319.4	20.4	2.3	50.2	2.9	0.9	72.8	0.3	1.1

Table S9. Lack of genotoxic activity of **c8** at different concentrations (1, 5, 10, 50, 100 µg/plate). All the t/c values were much lower than those obtained using a positive control (sodium azide, 1 µg/plate).

	HU	C1	C2	C3	C4	C5	C6	C7	C8
(μg/plate)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)	(mM)
1	0.0333	0.0224	0.0228	0.0257	0.0290	0.0233	0.0226	0.0217	0.0206
5	0.1665	0.1120	0.1138	0.1284	0.1448	0.1164	0.1130	0.1083	0.1029
10	0.3330	0.2240	0.2276	0.2568	0.2895	0.2328	0.2260	0.2165	0.2058
50	1.6650	1.1198	1.1378	1.2839	1.4476	1.1642	1.1301	1.0826	1.0292
100	3.3300	2.2396	2.2755	2.5677	2.8953	2.3283	2.2603	2.1652	2.0585

Table S10. Concentrations tested for all the samples (1, 5, 10, 50, 100 μg/plate) related to the respective molar concentrations.