Research Article

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Synthesis of orotic acid derivatives and their effects on stem cell proliferation

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Abstract: Orotic acid, a natural product, is involved in many biological processes. Human mesenchymal stem cells (hMSCs) have the potential of self-renewable and proliferation. They are commonly isolated from the bone marrow aspirates of large bones. The osteogenic potential of these stem cells has been extensively exploited by scientists in the past to evaluate the performance of synthetic scaffolds developed for tissue engineering. In this study, *N*-arylhydrazone derivatives of orotic acid have been synthesized, and their potential as stimulators of human mesenchymal stem cells has been evaluated. Some of the analogs exhibit well to moderate effect on the proliferation rate.

Keywords: orotic hydrazide, arylhydrazone, mesenchymal stem cells, proliferation

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1 Introduction

Heterocycles and heterocyclic derivatives continue to serve as versatile compounds for the synthesis of various natural products owing to the presence of various chromophores in them [1–5]. Pyrimidine carboxylic acid, commonly known as orotic acid, is found in many naturally occurring products such as milk whey and serves as an intermediate in the biosynthesis of pyrimidine, which is an essential component of DNA and RNA. Moreover, orotic acid can enhance the cardiac output and aid in the recovery from heart failure. It can also behave as a growth stimulant in mammals and may assist in the absorption of calcium, magnesium, and other essential nutrients. Orotic acid has also been reported to reduce bilirubin levels in infants and is also useful for the treatment of gout. Many orotic acid analogs exhibit remarkable antitumor and antimicrobial activities. Some of them also serve as enzyme inhibitors, thereby attracting the attention of chemists and molecular biologists [6–9].

Hydrazones constitute an important class of compounds in organic syntheses owing to the presence of the azomethine group in this molecule. Hydrazones and hydrazides are one of the most useful synthetic intermediates for the synthesis of various molecules and possible drug candidates [10]. Owing to their synthetic importance and their inherent biological activity, hydrazone derivatives have been the focus of interest for many synthetic chemists and biologists for many years. Their pharmacological profiles include antimicrobial, antiviral, anticancer, and antiinflammatory activities. The bioactivities of the hydrazide–hydrazone analogs are not only limited to the core moiety but are also dependent on the substituents on the terminal nitrogen atom. It has been long known that the introduction of aromatic substituents to the heterocyclic system results in more biologically potent molecules [11–13].

Human stromal (mesenchymal) stem cells (hMSCs) are multipotent stem cells that are capable of transforming into mesoderm-type cells such as osteoblasts

and adipocytes. Hence, they are being introduced into clinical trials for tissue regeneration. Certain heterocyclic compounds have an immense potential to enhance stem cell proliferation, thereby allowing them to differentiate into other mature cell types. Thus, such compounds can assist in the stem cell therapy to aid in medical procedures such as heart and bone marrow transplants. Heterocyclic compounds have been designed and synthesized on a number of molecular platforms including substituted purines pyrimidines, quinazolines, pyrazines, pyrrolopyrimidine, pyrazolopyrimidine, pyridazines, and hydrazones, which lend an appropriate chemical concern to look into modulate complex cellular mechanism [14–18]. However, to the best of our knowledge, the effect of pyrimidine carboxylic acid on such processes has not yet been investigated.

In continuation to our interest in the hydrazone–hydrazide chemistry, herein, the preparation of *N*-arylhydrazone derivatives of the orotic acid and their potential in the hMSC proliferation have been described.

2 Materials and methods

2.1 Chemicals and instruments

All solvents and reagents were purchased from Aldrich Chemical Co. and were used as received. IR spectra were recorded on a PerkinElmer spectrum BX FT-IR spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument (500 and 125 MHz, respectively) in DMSO-d_6. Mass spectra were obtained on a JEOL JMS-700 mass spectrometer, and the ionization method was electron ionization (70 eV). Melting points were measured with a Thermo Scientific 9100 apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed with fluorescent silica gel HF_{254} plates (Merck) and visualized under UV 254 UV spectrometer on charring with the EtOH–H_2SO_4 (5:1) system. Merck silica gel 60 (230–400 mesh) was used for column chromatography.

2.1.1 General procedure for the synthesis of compounds 2 and 3

The synthesis was conducted according to the protocol given by Britikova [19]. Briefly, to a solution of 2 mmol orotic acid (**1**) in ethanol/butanol (50 mL), a catalytic amount of HCl was added. The resulting mixture was refluxed for 10 h with stirring, followed by in vacuo evaporation of the solvent. The solid obtained was washed several times with cold water; recrystallization in ethanol–water mixture and purification by column chromatography (10% ethylacetate–hexane) afforded compounds **2** and **3**.

2.1.2 Structure identification of ethyl 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (3)

Yield: 71%, m.p.: 176. Brown crystals, IR (KBr) (ν, cm^{-1}): 1,715, 1,730 (C═O), 2,990 (NH), 3,335 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 1.30 (t, 3H, CH_3), 4.31 (m, 2H, CH_2), 6.05 (s, 1H, CH), 11.14 (br s, 1H, NH), 11.39 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 15.21, 62.2, 103.50, 142.09, 151.20, 163.05, 164.55. MS (70 eV): m/z (I_{rel}, %) 185 (71) $[M + H]^+$, 112 (100): calculated for $C_7H_8N_2O_4$ (184.05): HRMS: 184.0499.

2.1.3 General procedure for the synthesis of arylhydrazones 5, 7, 8, 10, 11, and 12

Compound **4** was synthesized from the reaction of ethyl ester with hydrazine hydrate in refluxing ethanol. A mixture containing the orotic hydrazide **4** (1 mmol) and an appropriate aromatic aldehyde (1.1 mmol) with a catalytic amount of acetic acid was heated under reflux and stirring for 3 h in ethanol. After completion of the reaction, as indicated by TLC, the reaction mixture was poured into crushed ice. The solid separated was filtered under suction, washed with ice-cold water (50 mL), passed through a short column (1:1 ethylacetate–hexane), and subsequently dried to afford the pure products.

2.1.4 Structure identification of (*E*)-*N*-(4-methoxybenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (5)

Yield: 64%, m.p.: 183°C. White solid, IR (KBr) (ν = cm^{-1}): 1,720, 1,723 (C═O), 3,019 (NH), 3,310 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 3.82 (s, 3H, OCH_3), 5.94 (s, 1H, CH), 7.70 (d, 2H, j = 8.5 Hz, arom), 7.83 (d, 2H, j = 9.0 Hz, arom), 8.33 (s, 1H, CH), 10.20 (br s, 1H, NH), 10.77 (br s, 1H, NH), 11.26 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 55.84, 100.79, 114.92, 125.37, 126.67, 129.57, 130.45, 150.38, 151.10, 151.30,

160.96, 164.51. MS (70 eV): m/z (I_{rel}, %) 289 (39) $[M + H]^+$, 267 (25), 133 (100): calculated for $C_{13}H_{12}N_4O_4$ (288.09): HRMS: 288.0819.

2.1.5 Structure identification of (*E*)-*N*′-(2,6-dichlorobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (7)

Yield: 69%, m.p.: 211°C. Light yellow solid, IR (KBr) (ν = cm^{-1}): 1,718, 1,723 (C═O), 3,029 (NH), 3,290 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 6.16 (s, 1H, CH), 7.49–7.63 (m, 3H, arom), 7.99, 8.62 (s, 1H, CH), 11.03 (br s, 1H, NH), 11.37 (br s, 1H, NH), 12.38 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 101.29, 129.71 (2×), 130.18, 132.78, 133.11, 134.64, 137.63, 148.91, 157.08, 164.46, 167.07. MS (70 eV): m/z (I_{rel}, %) 327 (39) $[M + H]^+$, 292 (25), 170 (100): calculated for $C_{12}H_8Cl_2N_4O_3$ (326.00): HRMS: 326.0736.

2.1.6 Structure identification of (*E*)-*N*-(4-nitrobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (8)

Yield: 69%, m.p.: 169°C. White solid, IR (KBr) (ν = cm^{-1}): 1,715, 1,723 (C═O), 3,039 (NH), 3,290 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 5.94 (s, 1H, CH), 7.83 (s, 1H, arom), 8.34 (m, 3H, CH, 2× arom), 8.74 (s, 1H, arom), 10.12 (br s, 1H, NH), 10.72 (br s, 1H, NH), 11.26 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 103.0, 123.16, 131.16 (2×), 134.91 (2×), 144.20, 149.51, 151.33, 151.90, 164.16, 167.63. MS (70 eV): m/z (I_{rel}, %) 304 (41) $[M + H]^+$, 298 (78), 176 (100): calcd for $C_{12}H_9N_5O_5$ (303.06): HRMS: 303.0541.

2.1.7 Structure identification of (*E*)-*N*-(3,4-dichlorobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (10)

Yield: 55%, m.p.: 157°C. White solid, IR (KBr) (ν = cm^{-1}): 1,722, 1,723 (C═O), 3,031 (NH), 3,290 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 5.99 (s, 1H, CH), 7.73 (m, 2H, arom), 7.99 (s, 2H, arom), 8.38 (s, 1H, CH), 11.03 (br s, 1H, NH), 11.27 (br s, 1H, NH), 12.33 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 101.17, 127.55, 129.44, 130.60, 132.27, 133.36, 134.74, 135.61, 147.78, 157.67, 164.48, 166.82. MS (70 eV): m/z (I_{rel}, %) 289 (39) $[M - Cl]^+$, 267 (25), 133 (100): calcd for $C_{12}H_8Cl_2N_4O_3$ (326.00): HRMS: 326.0317.

2.1.8 Structure identification of (*E*)-*N*-(4-bromobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (11)

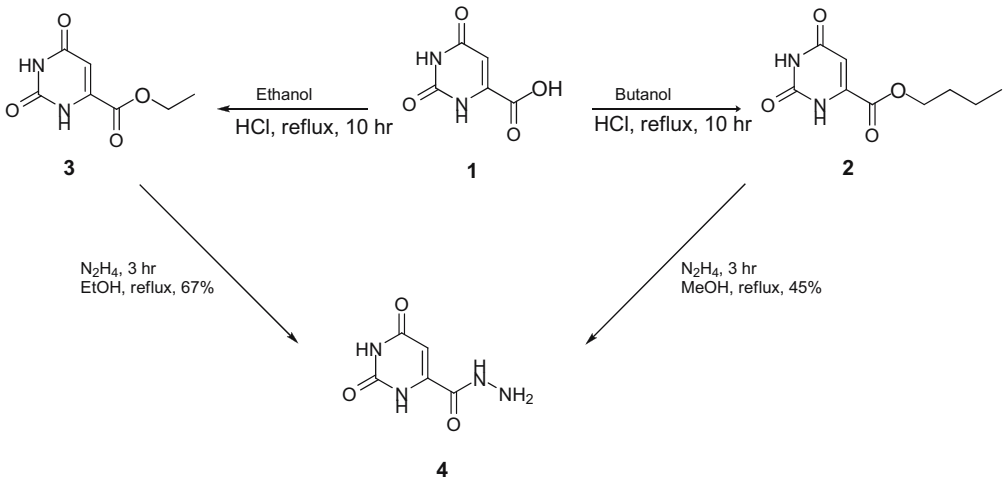
Yield: 59%, m.p.: 177°C. White solid, IR (KBr) (ν = cm^{-1}): 1,717, 1,725 (C═O), 3,050 (NH), 3,300 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 6.11 (s, 1H, CH), 7.65 (d, 2H, j = 8.5 Hz, arom), 7.83 (d, 2H, j = 8.5 Hz, arom), 8.37 (s, 1H, CH), 8.71 (br s, 1H, NH), 10.25 (br s, 1H, NH), 11.25 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 99.83, 127.41, 129.73, 130.69, 131.86, 132.10 132.50, 145.33, 150.88, 151.20, 164.56, 162.07. MS (70 eV): m/z (I_{rel}, %) 337 (30) $[M + H]^+$, 211 (90), 157 (28): calculated for $C_{12}H_9BrN_4O_3$ (335.99): HRMS: 335.9701.

2.1.9 Structure identification of (*E*)-*N*-(4-chlorobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carbohydrazide (12)

Yield: 63%, m.p.: 141°C. White solid, IR (KBr) (ν = cm^{-1}): 1,715, 1,730 (C═O), 2,990 (NH), 3,335 (OH). ^{1}H NMR (500.133 MHz, DMSO-d_6): δ = 6.10 (s, 1H, CH), 7.77 (d, 2H, j = 8.5 Hz, arom), 7.90 (d, 2H, j = 8.2 Hz, arom), 8.38 (s, 1H, CH), 8.71 (br s, 1H, NH), 10.13 (br s, 1H, NH), 11.26 (br s, 1H, NH). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ = 101.04, 128.97 (2×), 129.53 (2×), 130.50, 133.09, 145.04, 149.20, 151.28, 161.05, 164.48. MS (70 eV): m/z (I_{rel}, %) 276 (90) $[M - OH]^+$, 247 (45), 110 (100): calculated for $C_{12}H_9ClN_4O_3$ (292.04): HRMS: 292.0390.

2.1.10 X-ray crystallographic studies of compounds 2, 3, and 4

Crystals of compound **2** (Scheme 1) were obtained by crystallization from ethanol–water (3:1) via slow solvent evaporation. The crystallographic data for compound **2** have been deposited at the Cambridge Crystallographic Data Center (deposit CCDC 1483501). Brown needles of compound **3** (Scheme 1) were obtained by crystallization from ethanol–water via slow solvent evaporation. The crystallographic data for compound **3** have been deposited at the Cambridge Crystallographic Data Center (deposit CCDC 150815). Yellow crystals of compound **4** (Scheme 1) were obtained by crystallization from hot ethanol via slow solvent evaporation. The compound crystallized with two molecules of water in the crystal lattice. The crystallographic data for compound **4** have been deposited at the Cambridge Crystallographic Data Center (deposit CCDC 1483500).



Scheme 1: Reaction scheme for the synthesis of compounds **2**, **3**, and **4**.

2.2 Cell culture

The experiments were conducted using the previously established hTERT-MSC-CL1 (hMSC) cell lines. Cells from passages between 24 and 28 were used and were cultured in T75 culture flask (BD FalconTM, NJ, USA). Cells were monitored with an inverted light microscope (Observer A1, Zeiss®, Gottingen, Germany). hMSCs were grown in DMEM (Gibco, Cat No. 41966052) supplemented with 10% FBS (Gibco, Cat No. 26140087), 1% pen/strep (10,000 units of penicillin and 10,000 g of streptomycin/mL; Gibco, Cat No. 15140122), and 1% NEAA (X100; Gibco, Cat No. 11140035). After the cells reached 80–90% confluences in the culture flasks, they were trypsinized and transferred into falcon tubes. They were counted in the Neubauer hemocytometer counting chamber (PAUL MARIENFELD GMBH & CO.KG.). The cells were seeded at a density of 0.01×10^6 cells per well in a 96-well tissue culture plate. The following day, diluted compounds were added to the cells at the desired concentration in triplicates. Two days later, the media was changed to normal growth media. The next day was designated as day 1 of proliferation (Figure 1).

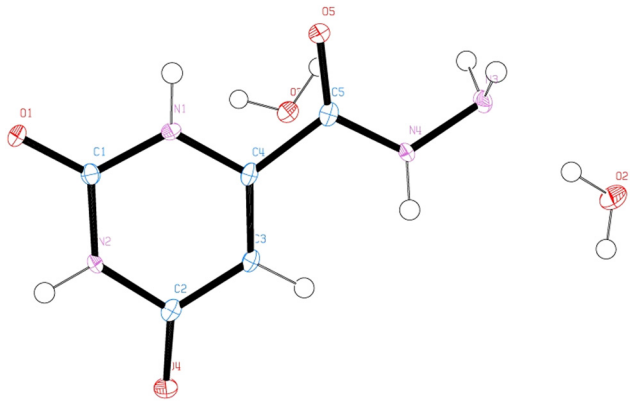


Figure 1: Crystal structure of compound **2**.

2.2.1 alamarBlue cell viability assay

Cell viability was determined using the alamarBlue assay using the protocol recommended by the manufacturer (AbD Serotec, Raleigh, NC, USA). Briefly, 100 µL cells were cultured in 96-well plates in the appropriate medium. At specific time points, 10 µL alamarBlue substrate was added, and the plates were incubated in the dark at 37°C for 1 h. The readings were subsequently taken in the fluorescence mode (Ex 530 nm/Em 590 nm) using BioTek Synergy II microplate reader (BioTek Inc., Winooski, VT, US).

Ethical approval: The conducted research is not related to either human or animal use.

3 Discussion

3.1 Synthesis of the target compounds

Owing to the low solubility in organic solvents, orotic acid has never been the choice as a starting material for synthetic chemists. Thus, very few reports are available on the synthesis of its analogs. To resolve this, orotic acid was esterified in butanol following a previously reported procedure. The product obtained (compound **2**)

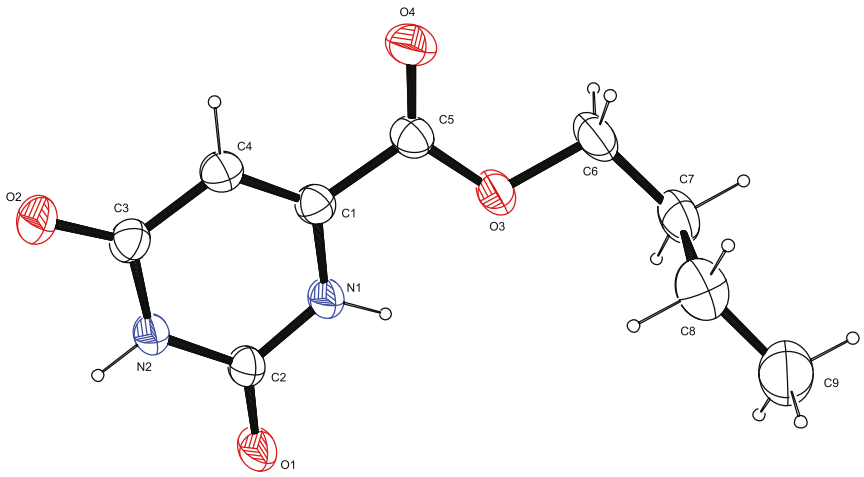


Figure 2: Crystal structure of compound **3**.

was recrystallized in ethanol–water (3:1) at room temperature to obtain crystals suitable for X-ray crystallography (structure is shown in Figure 2). However, the cumbersome workup, low yield, and toxicity restrict the wide application of this compound.

Considering the limitations of the reported methods, the need for the development of new and efficient methods is highly desirable. Therefore, ethanol was used along with the catalytic amount of HCl to afford **3** in good yield (Scheme 1). The solid obtained (compound **3**) was recrystallized in the ethanol–water mixture to afford crystals suitable for X-ray crystallographic studies (structure shown in Figure 2). The ethyl ester **3** was reacted with hydrazine hydrate in refluxing ethanol, yielding hydrazide **4** in 67% yield (Scheme 1). The same reaction was carried out with butyl ester of orotic acid, but the reaction time was longer, and the yield was significantly low. Starting material **4** (Figure 3) was then subjected through a series of acid-catalyzed condensation reaction with the various substituted aromatic aldehyde to afford the target compounds in excellent yields. All the compounds **2–12** (Schemes 1 and 2) were isolated as (*E*)-isomers, as confirmed by gated-decoupling (GD) measurements. In the ^{1}H NMR spectra of the synthesized molecules, a signal of pyrimidine CH appeared at the characteristic position, and the aromatic protons also appeared in their respective regions. The IR spectra of all the synthesized compounds showed bands in the regions 3,050–3,350 (NH), 1,710–1,723 (C═O), and 1,600–1,680 (C═O), which are typical of an uracil ring. The mass and HRMS data are also in accord with the proposed structures of compounds **2–12**.

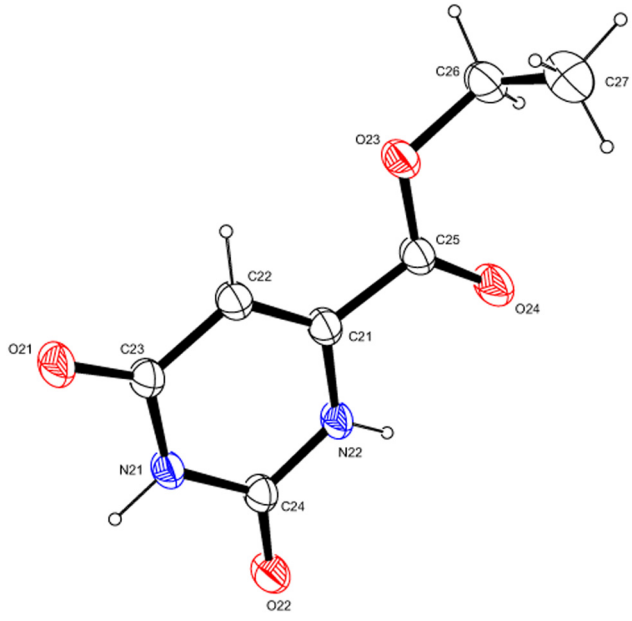


Figure 3: Crystal structure of compound **4**.

3.2 Effect of the synthesized compounds on stem cell proliferation

All the synthesized compounds were tested on hMSCs, and the results are listed in Tables 1–3. Cells were treated for 48 h with the test compounds in triplicates at concentrations ranging from 5.0 to 50 µg/mL. Moreover, the cells were incubated for an additional 3 days to ensure the effect of these compounds on hMSCs. It was observed that only after 1 day of treatment of the cells with high concentrations (500 µM) of most of the compounds reduced the proliferation significantly (Table 1). However, compounds **5**, **8**, **9**, **10**, **11**, and **12**

Table 1: Proliferation data for day 1

Day-1												
S. code	**500 µM**			**250 µM**			**50 µM**			**10 µM**		
	Avg	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**
DMSO	1096.0	9.6437		1208.3	16.8028		2676.0	137.1787		3791.3	135.019752	
1	1088.3	7.3711	0.3354	1620.3	10.0167	0.0000	3636.3	111.3927	0.0032	4793.3	116.6719	0.0006
2	1087.0	11.5326	0.3583	1167.3	98.3379	0.5159	2995.3	245.2944	0.2028	4250.7	177.5875	0.0235
3	1091.0	16.6433	0.6759	1389.3	87.8313	0.0248	3421.7	97.5209	0.0054	4329.5	202.9396	0.0351
4	1604.7	8.6217	2.7843	1830.3	91.5715	0.0003	3470.7	39.0043	0.0020	4612.3	207.1650	0.0045
5	5235.5	54.4472	0.0034	3579.5	95.4594	0.0865	2321.5	217.0818	0.0161	2496.5	102.5305	0.0029
6	1330.7	52.2143	0.0016	1567.7	64.7328	0.0007	3513.0	281.3343	0.0326	4303.0	241.8305	0.0512
7	1107.0	10.5357	0.2531	1154.7	72.0023	0.2771	2638.7	307.3375	0.8864	4010.3	200.1758	0.1913
8	3026.0	74.9533	0.7690	4577.5	553.6646	0.0656	5290.5	225.5671	0.0088	3899.0	130.3994	0.7647
9	5269.5	355.0000	0.0465	5590.5	325.9762	0.0121	5171.0	438.4062	0.0353	3823.5	1010.4556	0.8938
10	4924.0	132.9361	0.0068	2223.5	309.00566	0.1113	5593.5	62.9325	0.0008	2390.3	103.3650	0.0003
11	4214.5	152.0280	0.0195	5419.5	870.4484	0.0628	6973.5	833.6789	0.0285	5830.5	132.2290	0.0026
12	6425.0	425.6783	0.0092	4974.5	225.5671	0.0138	7222.0	450.0000	0.0430	5443.5	344.3610	0.0253

Stdv = standard deviation, *T* test = statistical test.

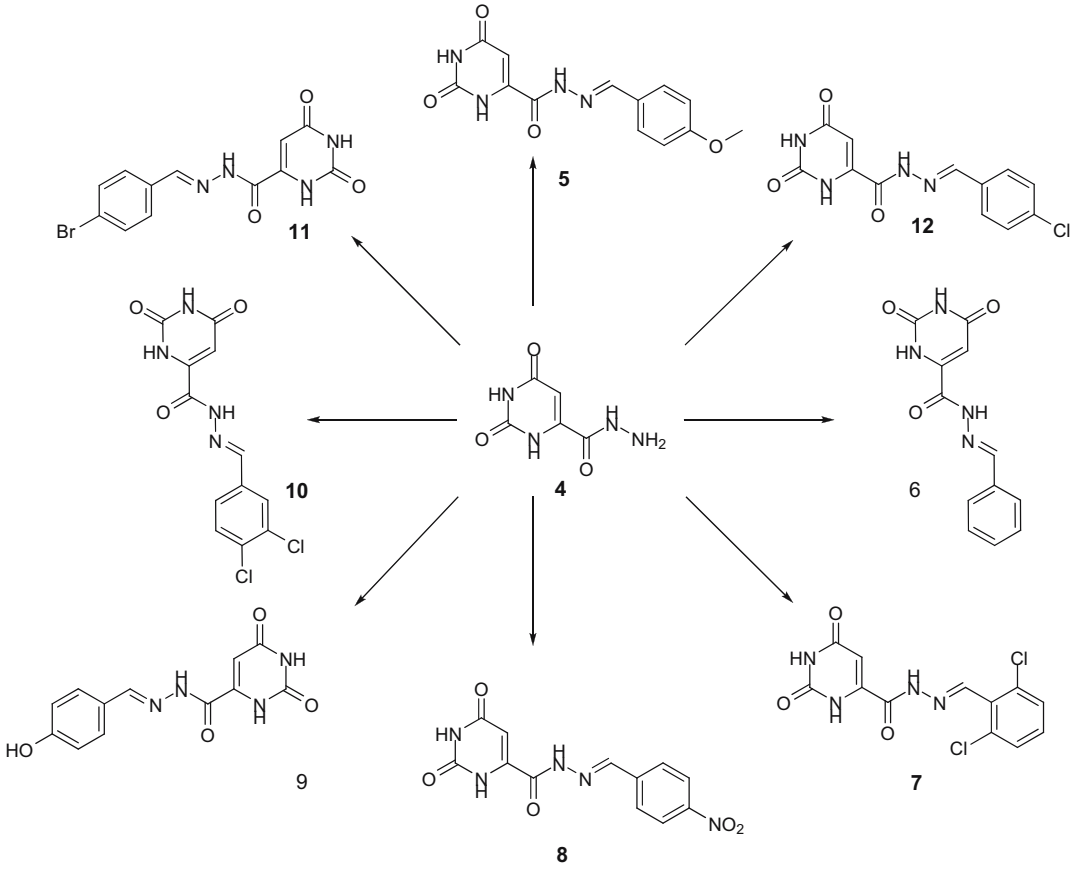
Table 2: Proliferation data for day 2

Day-2												
S. code	**500 µM**			**250 µM**			**50 µM**			**10 µM**		
	Avg	**Stdv**	***T* test**	**Avg.**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**
DMSO	999.0	10.5357		1062.3	21.5484		2522.7	213.0172		5044.3	142.8157	
1	1003.3	17.0392	0.7269	1186.0	52.4309	0.0195	3754.3	17.5594	0.0006	6972.7	197.8897	0.0002
2	1005.3	8.7369	0.4678	1055.3	15.2753	0.6701	3194.3	109.9879	0.0083	6445.7	250.5042	0.0011
3	1006.0	9.5394	0.4417	1143.3	64.7328	0.1089	3750.3	335.1005	0.0059	6089.0	177.6429	0.0014
4	1620.0	81.0617	0.0002	1556.3	53.2666	0.0001	3547.7	194.9803	0.0036	6578.7	592.6756	0.0121
5	18510.7	962.6548	0.0115	17558.7	1609.9920	0.0569	16434.7	1184.2645	0.7076	15428.0	1072.1842	0.1439
6	1243.7	52.9182	0.0014	1248.0	25.2389	0.0006	3685.7	197.5002	0.0023	6331.7	395.1460	0.0061
7	1011.7	4.0415	0.1238	1062.7	24.0069	0.9866	2455.0	442.7765	0.8232	6224.7	255.6645	0.0022
8	14936.0	1074.9246	0.8390	16152.7	1747.3564	0.2450	15853.3	451.0480	0.8309	18328.7	4978.4462	0.5989
9	21324.0	3071.9360	0.0284	25056.7	1324.8911	0.0005	25675.5	333.0473	0.0021	24465.5	1109.4505	0.0016
10	17977.0	3877.9794	0.2812	20272.3	1341.1522	0.0051	21647.7	1984.5504	0.0145	22522.0	1091.7729	0.0036
11	16545.7	1791.9714	0.2854	17391.0	1842.5767	0.0840	17395.7	1169.3820	0.2427	20079.3	3936.1282	0.2123
12	21559.0	5771.3375	0.1287	20456.7	5338.3457	0.1318	22833.5	642.7601	0.0066	23164.0	1938.8868	0.0096

were an exception, and these compounds increased the proliferation (Tables 1–3). In addition, these effects were more prominent at lower concentrations, at which these compounds (**6**, **9**, **10**, **11**, and **12**) imparted a significantly higher proliferation rate than the other compounds and compared to the control condition (DMSO). However, at lower concentrations, other compounds also showed significant upregulation such as **1**, **2**, and **3**. Only compound **7** did not have any effect on proliferation on day 1. A similar trend was observed on day 2; all the compounds at the highest concentration (500 µM) retarded the proliferation, while compounds **5**, **8**, **9**, **10**, **11**, and **12** (Scheme 2) still showed the significant higher proliferation rate. At lower concentrations (50 and 10 µM), all the compounds (except **7**) showed significant upregulation of proliferation. The same trend was seen on day 5, indicating that these compounds **5**, **8**, **9**, **10**, **11**, and **12** hold promising potential in the stem cell growth and possibly in differentiation (Tables 1–3). Other compounds

Table 3: Proliferation data for day 5

Day-5												
S. code	**500 µM**			**250 µM**			**50 µM**			**10 µM**		
	Avg	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**	**Avg**	**Stdv**	***T* test**
DMSO	1061.0	7.9373		1075.3	7.0238		5542.3	519.2161		15918.5	208.5965	
1	1068.0	4.0000	0.2442	1110.0	19.6723	0.0453	8124.3	510.1728	0.0036	19337.7	94.5004	0.0001
2	1073.0	5.0000	0.0911	1095.7	3.7859	0.0116	7473.3	854.2695	0.0287	17650.3	1565.9276	0.2361
3	1067.0	7.5498	0.3965	1105.0	11.0000	0.0170	8955.0	623.7171	0.0019	18102.3	312.3465	0.0034
4	1073.0	5.2915	0.0949	1121.3	10.9697	0.0036	7722.0	186.9679	0.0024	16321.0	2234.7611	0.8250
5	18430.5	2257.7920	0.2494	22953.0	2134.0483	0.0465	24550.0	33.9411	0.0000	29229.5	2322.8458	0.0618
6	1061.7	6.5064	0.9158	1093.3	0.5774	0.0115	8896.0	800.9295	0.0037	19469.3	2068.4178	0.1052
7	1050.7	4.0415	0.1149	1069.7	7.5719	0.3958	3934.3	791.9055	0.0423	18398.0	1486.6839	0.1123
8	23158.0	2262.1300	0.0230	23921.0	381.8377	0.0023	24148.5	1453.1044	0.0304	23016.0	1845.5487	0.8403
9	22249.5	443.3560	0.0050	22765.5	241.1234	0.0022	23440.0	234.7595	0.0011	25126.5	564.9783	0.0566
10	19910.5	311.8341	0.0094	18971.5	932.67384	0.0560	20063.5	982.1713	0.1379	25838.0	2129.8056	0.1841
11	23420.5	2089.5005	0.0374	21173.0	834.3860	0.0158	25119.0	1043.6896	0.0118	24440.0	1924.7447	0.3489
12	20446.5	400.9295	0.0088	20340.5	449.0128	0.0093	21494.0	1170.9688	0.0645	27037.5	999.1419	0.0354



Scheme 2: General synthesis of **5–12**. Reagents and conditions: ethanol, AcOH, reflux 5 h.

exhibited a less significant effect on proliferation, suggesting that the substituents play a crucial role in stem cell proliferation. Therefore, the compounds showing some upregulation in stem cell proliferation can be considered as promising leads for further investigation.

4 Conclusion

We have found that some orotic hydrazide derivatives showed a significant proliferation of mesenchymal stem cells at a lower concentration, but no change was observed at higher concentrations. Therefore, this study opens a new era of stem cell proliferation, and the exploration of more potent molecules can be achieved through further modifications.

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