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

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. 1H and 13C NMR spectra were recorded on a Bruker instrument (500 and 125 MHz, respectively) in DMSO-d6. 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 H254 plates (Merck) and visualized under UV 254 nm spectrometer on charring with the EtOH–H2SO4 (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) (v, cm⁻¹): 1,715, 1,730 (C=O), 2,990 (NH), 3,335 (OH). 1H NMR (500.133 MHz, DMSO-d6): δ = 1.30 (t, 3H, CH3), 4.31 (m, 2H, CH2), 6.05 (s, 1H, CH), 11.14 (br s, 1H, NH), 11.39 (br s, 1H, NH). 13C NMR (125.76 MHz, DMSO-d6): δ = 15.21, 62.2, 103.50, 142.09, 151.20, 164.55. MS (70 eV): m/z ([M + H]+, 112 (100); calculated for C7H8N2O4 184.0499): HRMS: 184.0499.

2.1.3 General procedure for the synthesis of aryldiazones 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-carboxyhydrazide (5)

Yield: 64%, m.p.: 183°C. White solid, IR (KBr) (v, cm⁻¹): 1,720, 1,723 (C=O), 3,019 (NH), 3,310 (OH). 1H NMR (500.133 MHz, DMSO-d6): δ = 3.82 (s, 3H, OCH3), 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). 13C NMR (125.76 MHz, DMSO-d6): δ = 55.84, 100.79, 114.92, 125.37, 126.67, 129.57, 130.45, 150.38, 151.00, 151.30.
160.96, 164.51. MS (70 eV): m/z (Irel, %) 289 (39) [M + H]+, 267 (25), 133 (100): calculated for C12H9BrN4O3 (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-carboxyhydrazide (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). ¹H NMR (500.133 MHz, DMSO-d₆): 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). ¹³C NMR (125.76 MHz, DMSO-d₆): δ = 101.29, 129.71 (2×), 130.18, 132.78, 133.11, 134.67, 148.91, 157.08, 164.46, 167.07. MS (70 eV): m/z (Irel, %) 327 (39) [M + H]+, 292 (25), 170 (100): calculated for C12H9Cl2N4O3 (326.00): HRMS: 326.0736.

2.1.6 Structure identification of (E)-N-(4-nitrobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxyhydrazide (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). ¹H NMR (500.133 MHz, DMSO-d₆): 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). ¹³C NMR (125.76 MHz, DMSO-d₆): 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 (Irel, %) 304 (41) [M + H]+, 298 (78), 176 (100): calculated for C12H9N4O3S (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-carboxyhydrazide (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). ¹H NMR (500.133 MHz, DMSO-d₆): 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). ¹³C NMR (125.76 MHz, DMSO-d₆): 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 (Irel, %) 289 (39) [M – Cl]+, 267 (25), 133 (100): calculated for C12H7Cl2N4O3 (326.00): HRMS: 326.0317.

2.1.8 Structure identification of (E)-N-(4-bromobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxyhydrazide (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). ¹H NMR (500.133 MHz, DMSO-d₆): 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). ¹³C NMR (125.76 MHz, DMSO-d₆): 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 (Irel, %) 337 (30) [M + H]+, 211 (90), 157 (28): calculated for C12H9BrN4O3 (335.99): HRMS: 335.9701.

2.1.9 Structure identification of (E)-N-(4-chlorobenzylidene)-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxyhydrazide (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). ¹H NMR (500.133 MHz, DMSO-d₆): 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). ¹³C NMR (125.76 MHz, DMSO-d₆): 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 (Irel, %) 276 (90) [M – OH]+, 247 (45), 110 (100): calculated for C12H9ClN4O3 (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).
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 flasks (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 $0.01 \times 10^6$ 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).

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)
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 1H 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.

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

Figure 2: Crystal structure of compound 3.

Figure 3: Crystal structure of compound 4.
Table 1: Proliferation data for day 1

| S. code | 500 µM | Avg | Stdv | T test | 250 µM | Avg | Stdv | T test | 50 µM | Avg | Stdv | T test | 10 µM | 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 | 1620.3 | 10.0167 | 3636.3 | 111.3927 | 4793.3 | 116.6719 | 0.0006 |
| 2       | 1087.0| 11.5326 | 1167.3 | 98.3379 | 2995.3 | 245.2944 | 4250.7 | 177.5875 | 0.0235 |
| 3       | 1091.0| 16.6433 | 1389.3 | 87.8313 | 3421.7 | 97.5209 | 4329.5 | 202.9396 | 0.0351 |
| 4       | 1604.7| 8.6217 | 1830.3 | 91.575 | 3470.7 | 39.0043 | 4612.3 | 207.1650 | 0.0045 |
| 5       | 5235.5| 54.4472 | 3579.5 | 95.4594 | 2321.5 | 217.0818 | 2496.5 | 102.5305 | 0.0029 |
| 6       | 1330.7| 52.2143 | 1567.7 | 64.7328 | 3513.0 | 281.3343 | 430.3 | 241.8305 | 0.0512 |
| 7       | 1107.0| 10.5357 | 1154.7 | 72.0023 | 2638.7 | 307.3375 | 4010.3 | 200.1758 | 0.1913 |
| 8       | 3026.0| 74.9533 | 4577.5 | 553.6646 | 5290.5 | 225.5671 | 3899.0 | 130.3994 | 0.7647 |
| 9       | 5269.5| 355.0000 | 5590.5 | 325.9762 | 5171.0 | 438.4062 | 3823.5 | 100.4565 | 0.8938 |
| 10      | 4924.0| 132.9361 | 2223.5 | 309.0056 | 5593.5 | 62.9325 | 2390.3 | 103.3650 | 0.0003 |
| 11      | 4214.5| 152.0280 | 5419.5 | 870.4484 | 6973.5 | 833.6789 | 5830.5 | 132.2290 | 0.0026 |
| 12      | 6425.0| 425.6783 | 4974.5 | 225.5671 | 3423.5 | 17.5594 | 5443.5 | 344.3610 | 0.0253 |

Stdv = standard deviation, T test = statistical test.

Table 2: Proliferation data for day 2

| S. code | 500 µM | Avg | Stdv | T test | 250 µM | Avg | Stdv | T test | 50 µM | Avg | Stdv | T test | 10 µM | 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 | 1186.0 | 52.4309 | 3754.3 | 17.5594 | 6972.7 | 197.8897 | 0.0002 |
| 2       | 1005.3| 8.7369 | 1055.3 | 15.2753 | 3194.3 | 194.8030 | 6445.7 | 250.5042 | 0.0011 |
| 3       | 1006.0| 9.5394 | 1143.3 | 64.7328 | 3750.3 | 335.1005 | 6089.0 | 177.6429 | 0.0014 |
| 4       | 1620.0| 81.6017 | 1556.3 | 53.2666 | 3547.7 | 94.9803 | 6587.8 | 592.6756 | 0.0121 |
| 5       | 18510.7| 962.6548 | 17558.7 | 1609.9920 | 16434.7 | 1184.2645 | 15428.0 | 1072.1842 | 0.0036 |
| 6       | 12437| 52.9182 | 1248.0 | 25.2389 | 3685.7 | 197.5002 | 6331.7 | 395.1460 | 0.0061 |
| 7       | 1011.7| 4.0435 | 1062.7 | 24.0609 | 2455.0 | 442.7765 | 6224.7 | 255.6645 | 0.0022 |
| 8       | 14936.0| 1074.9246 | 16152.7 | 1764.3564 | 18583.3 | 451.0480 | 18328.7 | 4978.4462 | 0.5989 |
| 9       | 21324.0| 3071.9360 | 25056.7 | 132.8911 | 25675.5 | 339.0473 | 24665.5 | 1109.4505 | 0.0016 |
| 10      | 17977.0| 3877.9794 | 20272.3 | 1341.1522 | 21647.7 | 198.5504 | 22522.0 | 1091.7729 | 0.0036 |
| 11      | 16545.7| 1791.9714 | 17391.0 | 1842.5767 | 17395.7 | 1169.3820 | 20079.3 | 3936.1282 | 0.2123 |
| 12      | 21559.0| 5771.3375 | 20456.7 | 5338.3457 | 22833.5 | 642.7601 | 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
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.

![Scheme 2: General synthesis of 5–12. Reagents and conditions: ethanol, AcOH, reflux 5 h.](image)

Table 3: Proliferation data for day 5

| Day 5 | S. code | 500 µM | 250 µM | 50 µM | 10 µM |
|-------|---------|--------|--------|-------|-------|
| Ave.  | Stdv.  | Ave.  | Stdv.  | Ave.  | Stdv.  | Ave.  | Stdv.  |
| DMSO  | 1061.0 | 7.924 | 1075.3 | 8.023 | 5542.3 | 519.2 | 15918.5 | 208.5 |
| 1     | 1068.0 | 4.000 | 1110.0 | 19.672 | 8124.3 | 510.1 | 19337.7 | 94.5  |
| 2     | 1073.0 | 5.000 | 1195.7 | 3.786 | 7473.3 | 854.3 | 1650.0 | 1565.9 |
| 3     | 1070.0 | 5.949 | 1105.0 | 11.000 | 6377.7 | 523.7 | 3218.7 | 2134.3 |
| 4     | 1073.0 | 5.291 | 1121.3 | 10.967 | 722.0 | 186.9 | 16321.0 | 234.7 |
| 5     | 18430.5 | 2257.8 | 2295.0 | 234.0 | 24550.0 | 33.9 | 29229.5 | 23.2 |
| 6     | 1061.7 | 6.064 | 1093.3 | 0.577 | 8896.0 | 800.9 | 19469.3 | 2068.4 |
| 7     | 1050.7 | 4.014 | 1069.7 | 7.519 | 3934.3 | 791.0 | 18398.0 | 1486.6 |
| 8     | 23180.0 | 2262.1 | 2392.1 | 381.8 | 24148.5 | 1453.1 | 24906.0 | 1485.4 |
| 9     | 22249.5 | 443.3 | 2276.5 | 241.1 | 23440.0 | 234.7 | 25126.5 | 564.9 |
| 10    | 19910.5 | 311.8 | 1897.1 | 932.6 | 20063.5 | 982.1 | 25838.0 | 2129.8 |
| 11    | 23420.5 | 2089.5 | 2117.3 | 834.3 | 25190.0 | 1043.6 | 23440.0 | 1924.7 |
| 12    | 20446.5 | 400.9 | 2034.0 | 449.0 | 21694.0 | 1170.1 | 27037.5 | 999.1 |

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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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Conflict of interest: The authors declare that they have no conflict of interest.

References

[1] Kucukguzel SG, Rollas S, Kucukguzel I, Kiraz M. Synthesis and antimycobacterial activity of some coupling products from 4-aminobenzozic acid hydrazones. Eur J Med Chem. 2009;34:1093–1100. doi: 10.1016/S0223-5234(99)00129-4.

[2] Dogan HN, Duran A, Rollas S, Sener G, Armutak Y, Keyer-Uysal M. Synthesis and structure elucidation of some new hydrazones and oxadiazolines: anticlotting activities of 2-(3-acetyloxy-2-naphthyl)-4-acetyl-5-substituted-1,3,4-oxadiazolines. Med Sci Res. 1998;26:755–8. doi: 10.1016/s0968-0896(02)00143-8.

[3] Bari A, Amer A, Syed SA, Azmak K, Al-obaid A. A facile one pot synthesis and anticancer evaluation of novel substituted 1,2-dihydropyridine and 1,2,3,4-tetrahydropyrimidine analogues. J Heterocycl Chem. 2016;53(2):377–82. doi: 10.1002/jhet.2400.

[4] Brown DJ, Cowden WB. Unfolded heterobicycles as amplifiers of phleomycin. VII. Some triazolyl-thiadiazoloyl- and oxadiazoloyl-pyridines and related pyrimidines. Austral J Chem. 1983;36:1469–74. doi: 10.1071/CH9812629.

[5] Craig H, Mark SW, Raymond SF. Anti-HIV-1 activity, toxicity, and stability studies of representative structural families of polynoxometalates. J Med Chem. 1990;33(10):2767–72. doi: 10.1021/jm00172a014.

[6] Matunas R, Lai AJ, Lee C. Iridium-catalyzed selective N-allylation of hydrazines. Tetrahedron. 2005;61:6298–308. doi: 10.1016/j.tet.2005.03.105.

[7] Bari A, Syed SA, Hashmi IA. Synthetic studies on the synthesis of some new heterocyclic compounds derived from 3-formylchromones. Chem Heterocycl Comdp. 2014;49(12):1723–30. doi: 10.1007/s10593-014-1424-4.

[8] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7. doi: 10.1126/science.284.5411.143.

[9] Hong C1, Plantado C. Potential anticancer agents. IV. 5-Substituted pyrimidine-6-carboxaldehydes and derivatives. J Med Chem. 1968;11(6):1182–90. doi: 10.1021/jm00312a018.

[10] Laxinunaryena E, Kumar T, Shivashankar SK, Chary S, Thirumala M. An efficient and clean synthesis of N-aryliden-6-hydroxy-2-methylpyrimidine-4-carboxyhydrazides. Der Pharma Chemica. 2011;3(3):149–55, https://www.derpharmachemica.com/pharma-chemica/an-efficient-and-clean-synthesis-offarylidene6hydroxy2methylpyrimidine4carboxyhydrazides.pdf.

[11] Gemma S, Kukreja G, Fattorusso C, Persico M, Romano M, Altarelli M, et al. Synthesis of N1-aryliden-N2-quinoyl- and N2 acrydihydrazone as potent antimalarial agents active against CQ-resistant P. falciparum strains. Bioorg Med Chem Lett. 2006;16:5384–8. doi: 10.1016/j.bcmcl.2006.07.060.

[12] Savini L, Chiassier, L, Travagli V, Pellerano C, Novellino E, Cosentino S, et al. New α-(N)-heterocyclyclidrazones: evaluation of anticancer, anti-HIV and antimicrobial activity. Eur J Med Chem. 2004;39:113–22. doi: 10.1016/j.ejmech.2003.09.012.

[13] Silva AG, Zapata-Suto G, Kummerle AE, Fraga CA, Barreiro EJ, Sudo RT. Synthesis and vasoactivity of new N-acylhydrazone derivatives, designed as LASSBio-294 analogues. Bioorg Med Chem. 2005;13:3431–7. doi: 10.1016/j.bmcl.2005.03.003.

[14] García QF, Posada OM, Pérez DG, Castro NH, Sarassa CA, Hansford DJ, et al. Isolation of human bone marrow mesenchymal stem cells and evaluation of their osteogenic potential. Rev Ingeniería Biomédica. 2008;23:48–55, http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S190997622008000100008&lng=es.

[15] Caroline A, Hubner R, Beller M, Frech MJ. Small molecules in stem cell research. Curr Pharm Biotech. 2013;14:36–45. doi: 10.2174/1389201013401000007.

[16] Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. Nature. 2008;453:519–23. doi: 10.1038/nature06968.

[17] Maes S, Shirasawa S, Yoshie S, Sato F, Kanoh Y, Ichikawa H, et al. Combination of small molecules enhances differentiation of mouse embryonic stem cells into intermediate mesoderm through BMP-7 positive cells. Biochem Biophys Res Commun. 2010;393:877–82. doi: 10.1016/j.bbrc.2010.02.111.

[18] McLean AB, D’Amour KA, Jones KL, Krishnamoorthy M, Kulik MJ, Reynolds DM, et al. Activin a efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed. Stem Cell. 2007;25:29–33. doi: 10.1634/stemcells.2006-0219.

[19] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7. doi: 10.1126/science.284.5411.143.