

Convenient synthesis of 1,3-disubstituted-2-thioxo-imidazolidin-4-ones as potential anti-tumor agents

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Abstract

1-(Arylidene) amino-2-thioxo-imidazolidine-4-ones (**3a,b**) have been synthesized via cyclization of 1-(arylidene) amino-3-(chloroacetyl) thiourea (**2a,b**) in ethanol in presence of fused sodium acetate under heating. Acetylation of compounds (**3a,b**) with acetic anhydride yielded the corresponding 1-(arylidene) amino-2-thioxo-3-acetylimidazolidin-4-ones (**4a, b**). Condensation of compounds (**3a,b**) with aromatic aldehydes in presence of piperidine yielded the corresponding aryl-[1-(arylidene)amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanols (**6a-d**). Halogenation of 1-(phenylethylidene) amino-2-thioxoimidazolidin-4-one (**3b**) with one mole of bromine produced N-bromo- 2- thioxoimidazolidin-4-one (**9**), while brominating 1-(benzylidene) amino-2-thioxoimidazolidin-4-one (**3a**) with two mole of bromine gave the corresponding 3, 5-dibromo-2-thioxoimidazolidin-4-one derivative (**10**). The characterizations of all synthesized compounds were done by elemental analysis and spectral studies. Moreover, the cytotoxic activities of the synthesized compounds were evaluated against human hepatocellular carcinoma cell line (HePG2) using MTT viability test. The results showed that the investigated compounds have a significantly cytotoxic effect.

Keywords: imidazolidine, thiourea, thiohydantoin and anticancer evaluation against HePG2 cell lines.

1. Introduction

2-Thioxoimidazolidin-4-ones represents an important pharmacore, which is present in various biologically active compounds. They have been reported to be of biological importance as anticonvulsant [1], antagonists of the thrombin receptor [2], antiviral [3], antidiabetic [4], potential anti-tumor agents [5-10]. They are also used in treatment of vascular dysfunction [11] and as inhibitor of a fatty acid amide hydrolase [12]. The 1-aminohydantoin [13,14] is an antimicrobial drug for treatment of urinary tract infections, while its analog dantrolene represents a well-known skeletal muscle relaxant.

In the previous papers [15-19] the syntheses of 2-thiohydantoin derivatives from arylidene thiosemicarbazones (**1**) with ethyl chloroacetate in the presence of fused sodium acetate in ethanol has been reported. As an extension of the previous work, this paper describes the syntheses of 1,3-disubstituted-2-thiohydantoins through different way starting from arylidene thiosemicarbazones (**1**) with ethyl chloroacetate in presence of acetic acid where it gives first 1,3-disubstituted thiourea which cyclized to 1,3-disubstituted-2-thiohydantoins, that used for preparation of new 1,3-disubstituted-2-thiohydantoins. The new synthesized compounds were evaluated as anticancer agents using different cell lines such as human hepatocellular carcinoma cell line (HePG2) using MTT viability test.

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2. Experimental section

2.1 Chemistry

The IR spectra of the synthesized compounds were taken on a Shimadzu FT spectrometer with a device of singly perturbed internal reflection. ^1H NMR spectra (in DMSO- d_6) were recorded on Bruker Ac-400 ultra-shield NMR spectrometer at 400 MHz, using TMS as internal standard. The ^{13}C NMR (500 MHz) spectra were run in dimethylsulfoxide (DMSO- d_6). Chemical shifts were related to that of the solvent. Mass spectra were obtained on a Joel JMSD-300 spectrometer operating at 70 eV. The elemental analysis was carried out on a perkin-Elmer C, H, N analyzer. Melting points were determined in open capillaries on a Gallenkamp melting point apparatus and are uncorrected.

1-(arylidene) amino-3-(chloroacetyl)thioureas (2a,b)

A solution of Arylidene thiosemicarbazones (**1**, 0.01mol) and ethyl chloroacetate(0.01mol) in glacial acetic acid (15ml) was heated under reflux for 30 minutes, then cooled. The resulting solid was filtered off, dried and recrystallized from ethanol to give compounds (**2a, b**).

1-(Benzylidene) amino-3-(chloroacetyl)thiourea(2a)

As yellow crystals, yield 73%, m.p.203°C. IR (KBr): 4378, 3253 (NH), 1710 (C=O), 1643 (C=N), 1448 cm^{-1} (C=S). ^1H NMR (DMSO- d_6): δ 4.03(s, 2H, COCH_2), 7.39-8.01 (m, 5H, Ar-H), 8.53 (s, 1H, CH=N), 11.42 (br. s, 1H, NH) and 11.98 (br. s, 1H, NHCO) ppm. ^{13}C NMR (DMSO- d_6) δ : 178.46 (C=S), 174.67 (C=O), 142.75 (HC=N), 134.65, 131.10, 129.29, 127.75 (C- aromatic) and 33.49 (CH_2Cl) ppm. MS: m/z (%) = 255.5 (M^+ , 26.4), 90 (100) Anal. Calcd. For $\text{C}_{10}\text{H}_{10}\text{N}_3\text{ClOS}$: C, 46.46; H, 3.91; N, 16.44; Cl, 13.89. Found: C, 46.31; H, 3.79; N, 16.21; Cl, 13.68.

1-(Phenylethylidene)amino-3-(chloroacetyl)thiourea(2b)

As yellow crystals, yield 71%, m.p. 178°C. IR(KBr): 3409, 3241 (NH), 1705 (C=O), 1639 (C=N), 1610, 1598 (C=C), 1461 cm^{-1} (C=S). ^1H NMR (DMSO- d_6): δ 2.34 (s, 3H, CH_3), 4.01 (s, 2H, COCH_2Cl), 7.38-7.89 (m, 5H, Ar-H), 11.43 (s, 1H, NH) and 11.99 (br. S, 1H, NHCO) ppm. ^{13}C NMR (DMSO- d_6) δ : 178.63 (C=S), 173.51 (C=O), 138.91 (C=N), 134.61, 131.05, 129.27, 128.11 (C-aromatic), 33.47 (CH_2Cl) and 14.61 (CH_3) ppm. MS: m/z (%) = 269.5 (M^+ , 35.6), 105(100) Anal. Calcd. For $\text{C}_{11}\text{H}_{12}\text{N}_3\text{ClOS}$: C, 48.98; H, 4.52; N, 15.58; Cl, 13.17. Found: C, 48.69; H, 4.33; N, 15.29; Cl, 13.03.

1-(Arylidene) amino-2-thioxo-imidazolidin-4-ones (3a,b)

A mixture of (**2**, 0.01mol) and fused sodium acetate (0.03mol) in ethanol (30ml) was heated under reflux for 4 hours, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compounds (**3a,b**).

1-(Benzylidene)amino-2-thioxo-imidazolidin-4-one (3a):

As pale yellow crystals, yield 73%, m.p. 239°C. IR(KBr): 3205 (NH), 1710 (C=O), 1643 (C=N), 1609, 1587 (C=C), 1444 cm^{-1} (C=S). ^1H NMR (DMSO- d_6): δ 3.93 (s, 2H, CH_2 -imidazolidine), 7.49-7.79 (m, 5H, Ar-H), 8.44 (s, 1H, CH=N) and 12.02 (s, 1H, NH- imidazolidine), ppm. ^{13}C NMR (DMSO- d_6) δ : 175.10 (C=S), 165.12 (C=O), 142.11 (HC=N), 135.13, 131.58, 129.77, 128.57 (C-aromatic) and 33.94 (CH_2 - imidazolidine), ppm. MS: m/z (%) = 219 (M^+ , 41.59), 90 (100) Anal. Calcd. For $\text{C}_{10}\text{H}_9\text{N}_3\text{OS}$: C, 54.79; H, 4.11; N, 19.18. Found: C, 54.61; H, 4.02; N, 19.01.

1-(Phenylethylidene) amino-2-thioxo-imidazolidin-4-one (3b):

As pale yellow crystals, yield 75%, m.p. 158°C. IR(KBr): 3213 (NH), 1712 (C=O), 1638 (C=N), 1611, 1592 (C=C), 1442 cm^{-1} (C=S). ^1H NMR (DMSO- d_6): δ 2.37 (s, 3H, CH_3), 3.86 (s, 2H, CH_2 -imidazolidine), 7.42-7.85 (m, 5H, Ar-H) and 11.94 (s, 1H, NH- imidazolidine), ppm. ^{13}C NMR (DMSO- d_6) δ : 173.86 (C=S), 161.20 (C=O), 137.76 (C=N), 131.20, 129.75, 128.37, 126.36 (C-aromatic), 32.76 (CH_2 -imidazolidine) and 14.06 (CH_3) ppm. MS: m/z (%) = 233 (M^+ , 55.7), 103 (100) Anal. Calcd. For $\text{C}_{11}\text{H}_{11}\text{N}_3\text{OS}$: C, 56.65; H, 4.72; N, 18.02. Found: C, 56.42; H, 4.56; N, 17.98.

1-(arylidene) amino-2-thioxo-3-acetylimidazolidin-4-ones (4a, b)

A solution of (**3**, 0.01mole) in acetic anhydride (20ml) was heated under reflux for 2 hours, then cooled. The solid formed was filtered off, washed with water, dried and purified by recrystallization from benzene to give (**4a, b**).

1-(Benzylidene)amino-2-thioxo-3-acetylimidazolidin-4-one (4a):

As pale yellow crystals, yield 69%, m.p. 143°C. IR(KBr): 1734, 1707 (C=O), 1638 (C=N), 1613, 1589 (C=C), 1446(C=S), 1027 (C-O) cm^{-1} . ^1H NMR (DMSO- d_6): δ 2.13 (s, 3H, COCH₃), 4.14 (s, 2H, CH₂-imidazolidine), 6.92-7.68 (m, 6H, Ar-H and H-imidazolidine), 8.31 (s, 1H, CH=N) and 12.13 (br. s, 1H, OH), ppm. ^{13}C NMR (DMSO- d_6) δ : 166.39 (C=S), 163.74 (C=O), 152.4 (=C-O), 136.02 (HC=N), 129.79, 129.06, 127.52, 126.41(C-aromatic), 39.81 (CH₂-imidazolidine), 21.50 (methyl of COCH₃) and 74.07 (N-CH of imidazolidine) ppm. MS: m/z (%) = 261 (M^+ , 1.75), 91 (100) Anal. Calcd. For C₁₂H₁₁N₃O₂S: C, 55.17; H, 4.23; N, 16.09. Found: C, 55.01; H, 4.07; N, 15.95.

1-(Phenylethylidene) amino-2-thioxo-3-acetylimidazolidin-4-one (4b):

As yellow crystals, yield 67%, m.p. 132°C. IR (KBr): 1732, 1704 (C=O), 1637 (C=N), 16108, 1593 (C=C), 1448 (C=S), 1023 (C-O) cm^{-1} . ^1H NMR (DMSO- d_6): δ 2.09 (s, 3H, COCH₃), 2.29 (s, 3H, N=C-CH₃), 4.34 (s, 2H, CH₂-imidazolidine), 7.11-7.60 (m, 6H, Ar-H and H-imidazolidine) and 11.91 (br. s, 1H, OH), ppm. ^{13}C NMR (DMSO- d_6) δ : 164.67 (C=S), 162.97 (C=O), 150.58 (=C-O), 137.87 (C=N), 128.73, 128.34, 125.92, 124.62 (C-aromatic), 40.32(CH₂-imidazolidine), 19.49 (CH₃), 75.21 (N-CH of imidazolidine) and 22.16 (methyl of COCH₃) ppm. MS: m/z (%) = 275 (M^+ , 26.28), 218 (100) Anal. Calcd. For C₁₃H₁₃N₃O₂S: C, 56.73; H, 4.73; N, 15.38. Found: C, 56.51; H, 4.59; N, 15.26.

Aryl-[1-(arylidene) amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanols (6a-d)

A mixture of (3, 0.01mol), aromatic aldehydes (such as benzaldehyde and 4-methoxy benzaldehyde) (0.01mol) and piperidine (2ml) in ethanol (50ml) was heated under reflux for 4 hours, the reaction mixture was cooled, poured into ice-water and neutralized by dil. acetic acid (10ml). The precipitate obtained was filtered off, washed with water, dried and recrystallized from ethanol to give (6a-d).

Phenyl-[1-(benzylidene)amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanol (6a):

As pale yellow crystals, yield 67%, m.p. 206°C. IR(KBr): 3390-2980 (br. OH), 1713 (C=O), 1647 (C=N), 1607, 1588 (C=C), 1460 (C=S) cm^{-1} . ^1H NMR (DMSO- d_6): δ 3.93 (s, 2H, CH₂ of imidazolidine ring), 7.11-7.84 (m, 11H, Ar-H and NCHO), 8.51 (s, 1H, CH=N) and 12.61 (br. S, 1H, OH) ppm. MS: m/z (%) = 325 (M^+ , 13.95), 42 (100) Anal. Calcd. For C₁₇H₁₅N₃O₂S: C, 62.77; H, 4.46; N, 12.77. Found: C, 62.61; H, 4.46; N, 12.77.

Phenyl-[1-(phenylethylidene)amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanol (6b):

As pale yellow crystals, yield 66%, m.p. 165°C. IR (KBr): 3380-2910 (br.OH), 1708 (C=O), 1638 (C=N), 1612, 1593 (C=C), 1468 (C=S) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 2.34 (s, 3H, CH₃), 3.87 (s, 2H, CH₂ of imidazolidine ring), 6.81-7.85 (m, 11H, Ar-H and NCHO) and 11.95 (br. S, 1H, OH) ppm. MS: m/z (%) = 339 (M^+ , 3.29), 249 (100) Anal. Calcd. For C₁₈H₁₇N₃O₂S: C, 63.72; H, 5.01; N, 12.39. Found: C, 63.58; H, 4.97; N, 12.17.

4-Methoxyphenyl-[1-(benzylidene)amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanol (6c):

As yellow crystals, yield 71%, m.p. 205°C. IR (KBr): 3390-2870 (br. OH), 1707 (C=O), 1638 (C=N), 1605, 1588 (C=C), 1451 (C=S) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 3.83 (s, 3H, OCH₃), 3.96 (s, 2H, CH₂ of imidazolidine ring), 7.07-8.21 (m, 10H, Ar-H and NCHO), 8.52 (s, 1H, CH=N) and 12.48 (br. S, 1H, OH) ppm. MS: m/z (%) = 355 (M^+ , 7.20), 134 (100) Anal. Calcd. For C₁₈H₁₇N₃O₃S: C, 60.84; H, 4.79; N, 11.83. Found: C, 60.67; H, 4.54; N, 11.61.

4-methoxyphenyl-[1-(phenylethylidene)amino-2-thioxo-4-oxo-imidazolidin-3-yl]carbanol (6d):

As yellow crystals, yield 67%, m.p. 212°C. IR (KBr): 3336-2870 (br.OH), 1708 (C=O), 1632 (C=N), 1608, 1593 (C=C), 1462 (C=S) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 2.37 (s, 3H, N=C-CH₃), 3.81 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂ of imidazolidine ring), 7.01-8.03 (m, 10H, Ar-H and NCHO) and 12.48 (br. S, 1H, OH) ppm. MS: m/z (%) = 369 (M^+ , 1.95), 40 (100) Anal. Calcd. For C₁₉H₁₉N₃O₃S: C, 61.79; H, 5.15; N, 11.38. Found: C, 61.56; H, 5.03; N, 11.09.

1-(Arylidene) amino-3-(aryl) acetoxymethyl-4-acetoxymidazolidin-2-thiones (7a-d)

A solution of carbanol derivatives (6, 0.01mol) in acetic anhydride (25ml), was heated under reflux for 2 hours, then cooled. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound (7a-d).

1-(Benzylidene) amino-3-(phenyl) acetoxymethyl-4-acetoxymidazolidin-2-thione (7a):

As pale yellow crystals, yield 63%, m.p. 185°C. IR (KBr): 1745, 1735 (C=O), 1643 (C=N), 1608, 1593 (C=C), 1466 (C=S), 1183, 1076 (C-O) cm^{-1} . ^1H NMR (DMSO- d_6) δ : 2.18 (s, 6H, 2COCH₃), 6.93-7.81 (m, 12H, Ar-H, H of imidazolidine ring and NCHO) and 8.41 (s, 1H, CH=N) ppm. ^{13}C NMR (DMSO- d_6) δ : 166.81 (C=S), 162.32, 162.01 (2C=O), 158.07 (C-O), 147.44 (C=N), 94.95 (NCHO), 135.55, 133.12, 132.22, 130.05, 129.98,

129.14, 127.69, 125.31 (C-aromatic), 74.81 (C-imidazolidine ring) and 21.57, 21.58 (C-methyl in 2COCH₃) ppm. MS: m/z (%) = 409 (M⁺, 1.48), 134 (100) Anal. Calcd. For C₂₁H₁₉N₃O₄S: C, 61.61; H, 4.64; N, 10.72. Found: C, 61.41; H, 4.24; N, 10.13.

1-(Phenylethylidene) amino-3-(pheny) acetoxymethyl-4-acetoximidazolidin-2-thione (7b):

As pale yellow crystals, yield 64%, m.p. 143°C. IR (KBr): 1743, 1732 (C=O), 1639 (C=N), 1609, 1596 (C=C), 1456 (C=S), 1123, 1087 (C-O) cm⁻¹. ¹HNMR (DMSO-d₆) δ: 2.17 (s, 6H, 2COCH₃), 2.35 (s, 3H, CH₃) and 6.98-7.91 (m, 12H, Ar-H, H of imidazolidine ring and NCHO) ppm. ¹³CNMR (DMSO-d₆) δ: 166.94 (C=S), 162.30, 161.89 (2C=O), 157.78 (C-O), 146.80 (C=N), 93.98 (NCHO), 134.96, 131.12, 129.23, 128.64, 128.92, 126.90, 126.81, 125.84 (C-aromatic), 74.83 (C-imidazolidine ring), 21.62, 21.63 (C-methyl in 2COCH₃), 19.62 (CH₃) ppm. MS: m/z (%) = 423 (M⁺, 8.95), 104 (100) Anal. Calcd. For C₂₂H₂₁N₃O₄S: C, 62.41; H, 4.96; N, 9.93. Found: C, 62.24; H, 4.79; N, 9.93.

1-(Benzylidene) amino-3-(4-methoxypheny) acetoxymethyl-4-acetoximidazolidin-2-thione (7c):

as yellow crystals, yield 67%, m.p. 130°C. IR (KBr): 1746, 1735 (C=O), 1642 (C=N), 1589 (C=C), 1463 (C=S), 1167, 1076 (C-O) cm⁻¹. ¹HNMR (DMSO-d₆) δ: 2.19 (s, 6H, 2COCH₃), 3.82 (s, 3H, OCH₃), 6.93-7.73 (m, 11H, Ar-H, H-imidazolidine ring and NCHO), 8.39 (s, 1H, CH=N) ppm. ¹³CNMR (DMSO-d₆) δ: 166.74 (C=S), 161.43, 161.01 (2C=O), 157.78 (C-O), 147.65 (C=N), 91.05 (NCHO), 74.74 (C-imidazolidine ring), 55.97 (OCH₃), 21.57, 21.56 (C-methyl in 2COCH₃), 103.04, 129.93, 129.13, 127.65, 125.54, 121.97, 115.44, 114.45 (C-aromatic). MS: m/z (%) = 439 (M⁺, 8.9), 43 (100). Anal. Calcd. For C₂₂H₂₁N₃O₅S: C, 60.14; H, 4.78; N, 9.57. Found: C, 60.03; H, 4.57; N, 9.33.

1-(Phenylethylidene) amino-3-(4-methoxypheny) acetoxymethyl-4-acetoximidazolidin-2-thione (7d):

As yellow crystals, yield 67%, m.p. 142°C. IR (KBr): 1747, 1734 (C=O), 1638 (C=N), 1611, 1593 (C=C), 1463 (C=S), 1167, 1083 (C-O) cm⁻¹. ¹HNMR (DMSO-d₆) δ: 2.17 (s, 6H, 2COCH₃), 2.36 (s, 3H, CH₃), 3.83 (s, 3H, OCH₃), 6.81-7.81 (m, 11H, H-imidazolidine ring and NCHO) ppm. ¹³CNMR (DMSO-d₆) δ: 166.90 (C=S), 160.09, 161.31 (2C=O), 157.25 (C-O), 145.8 (C=N), 87.15 (NCHO), 83.34 (C-imidazolidine ring), 55.46 (OCH₃), 22.01, 22.16 (C-methyl in 2COCH₃), 19.59 (CH₃), 137.61, 131.64, 131.58, 128.40, 128.43, 125.96, 125.05, 121.49 (C-aromatic). MS: m/z (%) = 453 (M⁺, 2.99), 77 (100). Anal. Calcd. For C₂₃H₂₃N₃O₅S: C, 60.93; H, 5.08; N, 9.27. Found: C, 60.76; H, 4.97; N, 9.02.

1-(Phenylethylidene) amino-2-thioxo-3-(N-bromo) imidazolidin-4-one 9:

A solution of (3b, 0.01mol) in glacial acetic acid (15ml) was stirred at room temperature then a bromine solution (0.01mol) in glacial acetic acid was added drop wise with stirring for 2 hours. The precipitate formed was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound **9** as pale yellow crystals, yield 72%, m.p. 200°C. IR (KBr): 1733 (C=O), 1638 (C=N), 1607, 1595 (C=C), 1447 (C=S) cm⁻¹. ¹HNMR (DMSO-d₆) δ: 2.35 (s, 3H, CH₃), 3.85 (s, 2H, H-imidazolidine ring), 10.52-10.59 (br. s, 1H, OH), 7.90-7.86 (m, 5H, Ar-H) ppm. ¹³CNMR (DMSO-d₆) δ: 173.94 (C=S), 164.18 (C=O), 160.32 (=C-O of imidazolidine ring), 137.78 (C=N), 129.81, 128.81, 126.40, 130.31 (C-aromatic), 32.86 (CH₂ of imidazolidine ring), 14.67 (CH₃) ppm. MS: m/z (%) = 311 (M⁺, 5.26), 77 (100). Anal. Calcd. For C₁₁H₁₀N₃BrOS: C, 42.44; H, 3.21; N, 13.05; Br, 25.40. Found: C, 42.19; H, 3.12; N, 13.33; Br, 25.25.

1-(Benzylidene) amino-2-thioxo-3-(N-bromo)-5-bromoimidazolidin-4-one 10

A solution of (3a, 0.01mol) in glacial acetic acid (20ml) was stirred at room temperature then a bromine solution (0.02mol) in glacial acetic acid was added drop wise with stirring for 2 hours. The precipitate formed was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound **10** as yellow crystals, yield 71%, m.p. 220°C. IR (KBr): 1731 (C=O), 1641 (C=N), 1609, 1589 (C=C), 1465 (C=S) cm⁻¹. ¹HNMR (DMSO-d₆) δ: 6.42 (s, 1H, H-imidazolidine ring), 7.47-7.83 (m, 5H, Ar-H), 8.49 (s, 1H, CH=N) ppm. ¹³CNMR (DMSO-d₆) δ: 174.21 (C=S), 163.17 (C=O), 82.31 (C of imidazolidine ring), 147.31 (HC=N), 135.61, 131.31, 129.72, 128.48 (C-aromatic) ppm. MS: m/z (%) = 375 (M⁺, 1.98), 164 (100). Anal. Calcd. For C₁₁₀H₇N₃Br₂OS: C, 32.00; H, 1.87; N, 11.20; Br, 42.13. Found: C, 31.88; H, 1.66; N, 11.02; Br, 42.01.

2.2 Pharmacology

2.2.1 *In vitro* studies

2.2.2 Cell lines

Human hepatocellular carcinoma (HepG-2) cells were obtained from the American type culture collection ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 500 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub cultured two to three times a week.

2.2.3 Cytotoxic assay of 2-thioxo-imidazolidin-4-one derivatives

The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 500 µg/ml gentamycin. The monolayers of 10000 cells adhered at the bottom of the wells in a 96-well micro titer plate incubated for 24 h. at 37°C in a humidified atmosphere with 5% CO₂. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of the test sample in the fresh maintenance medium and incubated at 37°C. A control of unreacted cells was made in the absence of the test sample. Six wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 490 nm using ELISA reader (Sun Rise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation.

The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as $[1 - (OD_t / OD_c \times 100\%)]$ where; OD_t is the mean optical density of wells treated with the test sample. OD_c is the mean optical density of unreacted cells.

The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effect in 50% of inactivated cells, was estimated from graphic plots.

2.2.4 Anti-tumor activity of 2-thioxo-imidazolidin-4-one derivatives

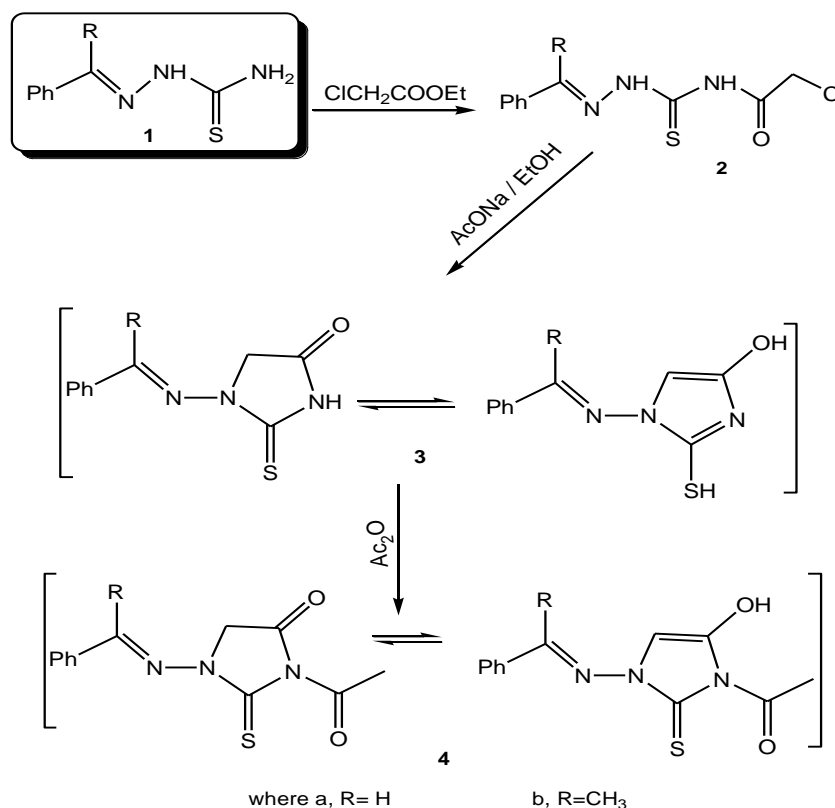
In this study, 2-thioxo-imidazolidin-4-one derivatives were evaluated for their human tumor cell growth inhibitory activity against Hepatocellular carcinoma (HePG-2). The measurements of cell growth and viability were determined as described in the Mossman (1983) and Vijayan et al (2004) method [20, 21]. Inhibitory activity against Hepatocellular carcinoma cells (HePG2 cell line) was tested by using different concentrations of the tested samples (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) and vinblastine drug as standard reference. The viability cells (%) were determined by colorimetric method. Inhibitory concentration fifty (IC₅₀) of prepared compounds of (HepG-2) cell line were calculated from **Tables 1, 2** and **Figures 1, 2** and **3**.

3. Results and discussion

The synthesis pathway leading to the title compounds is outlined in (Schemes 1 and 2). Arylidene thiosemicarbazones (**1**) are prepared via the condensation of benzaldehyde and / or acetophenone with thiosemicarbazide in ethanol. Treatment of arylidene thiosemicarbazones (**1a,b**) with ethyl chloroacetate in glacial acetic acid under reflux for thirty minutes led to the formation of 1-(arylidene)amino-3-(chloroacetyl) thiourea (**2a, b**).

The ¹H NMR spectra of compounds (**2a, b**) showed signals at 4.03 and 4.01 (2H) of methylene group (COCH₂Cl). Compound (**2b**) showed signal at 2.34 ppm (3H) for methyl group while compound (**2a**) showed a signal at 8.53 ppm due to the presence of methyne group (CH=N). In addition, the ¹H NMR spectra of compounds (**2a, b**) exhibited signals at 7.38 – 8.01 ppm (Ar-H) due to the presence of the phenyl groups, signals at 11.42, 11.43 ppm for (NH) group and 11.98, 11.99 ppm for (NHCO) group. The ¹³C NMR spectra of compounds (**2a, b**) showed two signals at 178.46, 178.63 ppm for thiocarbonyl group (C=S) and 174.67, 173.51 for the carbonyl group (C=O), in addition signals at 142.75, 138.91 for the (C=N) group have been appeared. Due to the presence of C- aromatic signals at 134.61-127.63 have been appeared, they also showed signals at 33.49, 33.47 ppm for methylene group (COCH₂Cl). The ¹³C NMR of compound (**2b**) showed signal at 14.61 ppm for methyl group (CH₃).

1-(Arylidene) amino-2-thioxoimidazolidin-4-ones (**3a,b**) were obtained via cyclization of 1,3-di substituted thiourea (**2a,b**) with heating in ethanol in the presence of fused sodium acetate.

Scheme1: synthetic routes of compounds **2**, **3** and **4**.

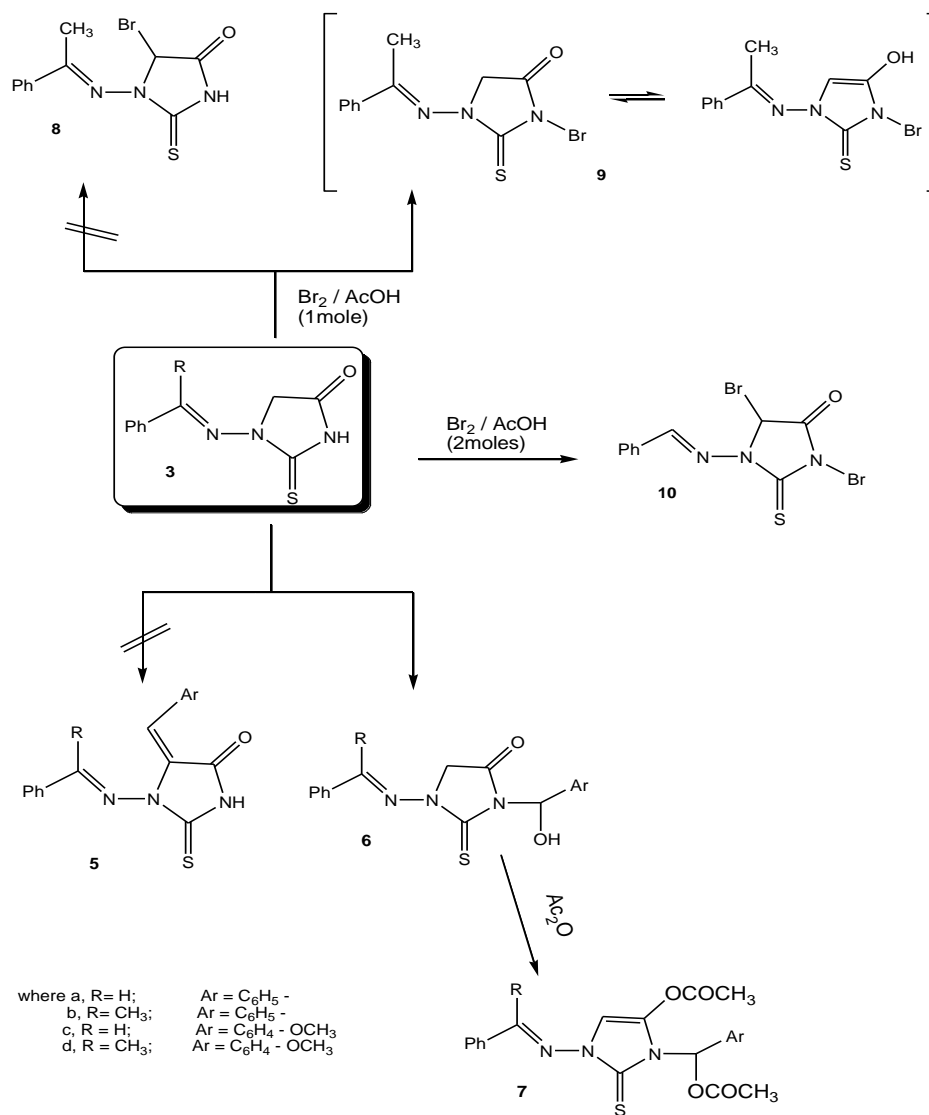
The mass spectra of compounds (**3a**, **b**) showed the expected molecular ion peaks at m/z 219 (M^+) and m/z 233 (M^+) respectively. The IR spectra of (**3a**, **b**) showed characteristic bands at 3205, 3213 cm^{-1} for (NH), 1710, 1712 cm^{-1} for (C=O), 1643, 1638 cm^{-1} for (C=N), 1444, 1442 cm^{-1} for (C=S).

The ^1H NMR spectra of compounds (**3a**, **b**) showed signals at 3.93, 3.86 ppm for methylene group (CH_2), 12.02, and 11.94 for (NH) of imidazolidine ring. Compound (**3a**) showed the characteristic signals of 1-(benzylidene)amino at 7.49-7.79ppm (m, 5H, Ar-H) and signal at 8.44ppm for ($\text{CH}=\text{N}$), whereas ^1H NMR spectrum of compound (**3b**) showed the expected signals of the aromatic proton at 7.42-7.85 (m, 5H, Ar-H), 2.37 (3H, CH_3) of the substituent 1-(phenylethylidene)amino.

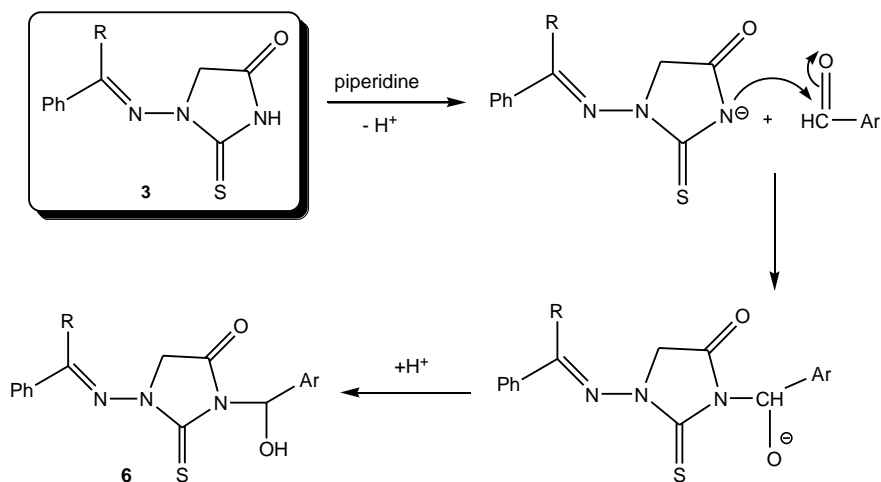
The ^{13}C NMR spectra of compounds (**3a**, **b**) have been showed characteristic signals at 175.10, 173.86 ppm for (C=S), 165.12, 161.20 for (C=O), signals at 33.94, 32.76 ppm for methylene group (CH_2) of imidazolidine ring. The substituent of 1-(benzylidene)amino in compound (**3a**) exhibited signals at 142.11 ppm of ($\text{CH}=\text{N}$) and 135.13, 131.58, 129.77, 128.57 ppm of (C-aromatic). 1-(phenylethylidene)amino substituent in compound (**3b**) exhibited signals at 137.76 for (C=N), 131.20, 129.75, 128.37, 126.36 ppm for (C-aromatic) and signal at 14.06 ppm for (CH_3).

Acetylation of 1-(arylidene) amino-2-thioxoimidazolidin-4-ones (**3a**, **b**) with acetic anhydride under reflux led to the formation of 1-(arylidene)amino-2-thioxo-3-acetylimidazolidin-4-ones (**4a**, **b**). The IR spectra of compounds (**4a**, **b**) showed the disappearance of (NH) group and appearance of bands at 1734, 1732 cm^{-1} for carbonyl of acetyl groups. The ^1H NMR spectra of compounds (**4a**, **b**) showed the absence of hydrogen protons of (NH) groups and appearance of signals at 2.13 and 2.09 ppm of hydrogen protons of methyl group, which obtained from acylation of compounds (**3a**, **b**). The ^{13}C NMR spectra of compounds (**4a**, **b**) showed appearance of new signals at 163.74 ppm, 162.97 ppm of carbonyl group (C=O) and 21.50 ppm, 22.16 ppm of carbon of methyl group (CH_3).

Condensation of 1-(arylidene) amino-2-thioxoimidazolidin-4-ones (**3a**, **b**) with aromatic aldehydes (namely benzaldehyde and 4-methoxy benzaldehyde) in ethanol in presence of piperidine under reflux gave the corresponding to aryl-[1-(arylidene)amino-2-thioxo-4-oxoimidazolidin-3-yl]carbanol (**6a**, **b**) which does not give the expected structure (**5**).

Scheme 2: Synthetic routes of compounds **6**, **7**, **9** and **10**

Compound (**6**) may be formed by the nitrogen nucleophilic attack at carbonyl group of aldehydes as shown in (Scheme 3).

Scheme 3: Reaction mechanism for the synthetic route of compound **6**.

The mass spectra of compounds (**6a-d**) showed the expected molecular ion peaks at m/z 325, 339, 355 and 369, respectively. The IR spectra of compounds (**6a-d**) showed abroad bands at $3380\text{--}2870\text{ cm}^{-1}$ due to the hydroxyl group (OH). The ^1H NMR spectra of compounds (**6a-d**) showed signals at 3.80-3.96 ppm for the two protons attributed to methylene group (CH_2) in imidazolidine ring, signals at 6.89-8.21 ppm multiplets due to the presence of phenyl protons and NCHO proton, and signals at 11.95-12.61 ppm due to the proton of hydroxyl group (OH). ^1H NMR spectra of compounds (**6a, c**) showed signals at 8.51 and 8.52 ppm assigned to proton of ($\text{CH}=\text{N}$) group. The spectra of compounds (**6c, d**) showed signals at 3.83 and 3.81 ppm due to the protons of methoxy group (OCH_3) in benzene ring, while the spectra of compounds (**6b, d**) showed signals at 2.34 and 2.37 ppm due to the protons of methyl group (CH_3).

Acylation of carbanol derivatives (**6a-d**) with acetic anhydride under reflux led to the corresponding 1-(arylidene) amino-3-(aryl) acetoxymethyl-4-acetoximidazolidine-2-thiones (**7a-d**). The IR spectra of compounds (**7a-d**) showed a medium intensity bands at $1747\text{--}1732\text{ cm}^{-1}$ assigned to the carbonyl acetoxyl groups and the disappearance of bands of hydroxyl group (OH). The characteristic bands at $1632\text{--}1645$ and $1445\text{--}1468\text{ cm}^{-1}$ due to ($\text{C}=\text{N}$) group and thiocarbonyl group ($\text{C}=\text{S}$) have been appeared. The ^1H NMR spectra of compounds (**7a-d**) showed signals at 2.16-2.19 ppm attributed to the protons of acetyl group (CH_3CO), signals at 6.81-8.01 ppm due to the presence of phenyl protons, imidazole proton and (NCHO). ^1H NMR spectra of compounds (**7a, c**) displayed signals at 8.41 and 8.39 ppm due to the ($\text{CH}=\text{N}$) group. ^1H NMR spectra of compounds (**7c, d**) displayed signals at 3.82 and 3.83 ppm of three protons of methoxy group (OCH_3). The spectra of compounds (**7b, d**) exhibited an intense signal at 2.35 and 2.37 ppm due to the methyl (CH_3) protons. The ^{13}C NMR spectra of compounds (**7a-d**) strongly supported the structure formation, as the signal at 32.85 which is assigned for (NCH_2CO) in imidazolidine ring was disappear and new signals at 74.81, 74.83, 74.74 and 83.34 ppm were observed due to the carbon of imidazolidine ring, the spectra also exhibited signals at 166.81, 166.94, 166.74 and 160.90 ppm due to the presence of thiocarbonyl group ($\text{C}=\text{S}$) in imidazolidine ring, signals at 162.32-160.09 due to the carbonyl of acetyl groups, signals at 135.55-113.87 ppm due to the presence of aromatic carbons, signals at 21.57-22.16 ppm for the methyl carbon (2COCH_3), 19.59-19.62 ppm for (CH_3), 55.46-55.97 for (OCH_3), 147.65-145.20 ppm for ($\text{C}=\text{N}$). The mass spectra of compounds (**7a-d**) showed intense molecular ion peaks at m/z 409, 423, 439, and m/z 453 consistent with the molecular formula $\text{C}_{21}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$, $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_4\text{S}$, $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_5\text{S}$ and $\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}_5\text{S}$ respectively.

Halogenation of 1-(phenylethylidene)amino-2-thioxoimidazolidin-4-one (**3b**), with one mole of bromine in glacial acetic acid at room temperature afforded the corresponding 1-(phenylethylidene)amino-2-thioxo-3-(N-bromo)imidazolidin-4-one (**9**), which doesn't give the expected resulting product 1-(phenylethylidene)amino-2-thioxo-5-bromoimidazolidin-4-one (**8**), while the treatment of 1-(benzylidene)amino-2-thioxo-imidazolidin-4-one (**3a**) with two mole of bromine in glacial acetic acid at room temperature yielded the corresponding 1-(benzylidene)amino-2-thioxo-3-(N-bromo)-5-bromoimidazolidin-4-one (**10**).

The ^1H NMR spectrum of compound (**9**) strongly supported the structure formation as the signal at 11.94 ppm assigned to NH of imidazolidine ring disappeared and a new broad signal at 10.52-10.59 ppm was observed attributed to the OH group, signal at 3.85 ppm of two protons of methylene group (CH_2) in imidazolidine ring has been appeared, which indicate that the compound (**9**) is in keto-enoltutomerism.

The ^{13}C NMR spectrum of compound (**9**) showed signals at 173.94 ppm for ($\text{C}=\text{S}$), 164.18 ($\text{C}=\text{O}$) and 160.32 ppm ($=\text{C}-\text{O}$ of imidazolidine ring). These are supported the presence of keto-enoltutomerism. The spectrum also showed signals at 137.78 for ($\text{C}=\text{N}$), signal at 32.86 ppm for carbon of methylene in imidazolidine ring, signal at 14.67 ppm for methyl group (CH_3) and signals at 129.81, 128.81, 126.40, 130.31 for the phenyl ring. The ^1H NMR spectrum of compound (**10**) showed signals at 7.47-7.783 ppm assigned to the five protons of phenyl ring, signal at 6.42 ppm for one proton of imidazolidine ring and disappearance of the signal at 3.93 ppm of the two protons of imidazolidine ring (NCH_2CO), it is also showed signal at 8.49 ppm of one proton of ($\text{CH}=\text{N}$) and disappearance of the signal at 12.02 ppm of NH of imidazolidine ring, which confirm the structure of compound (**10**).

The ^{13}C NMR spectrum of compound (**10**) showed signals at 174.21 ppm for thiocarbonyl group ($\text{C}=\text{S}$), 163.17 for the carbonyl group ($\text{C}=\text{O}$), 147.31 for ($\text{HC}=\text{N}$), 135.61, 131.31, 129.72, 128.48, for the carbons of phenyl ring and 82.31 for the carbon of imidazolidine ring.

Table 1: Evaluation of cytotoxicity of compounds (3a, 4a, and 6a-d) against HePG2 cell line

Sample conc. (μg)	Viability (%)							Vinblastine standard
	3a	4a	4b	6a	6b	6c	6d	
50	20	43.36	20.74	46.27	7.28	25.89	39.18	14.38
25	39.82	60.74	38.95	68.39	18.93	34.67	52.72	16.13
12.50	72.64	79.48	71.87	81.26	26.51	45.79	68.49	24.25
6.25	86.51	87.26	87.53	92.65	39.44	76.82	82.51	45.13
3.125	92.35	94.57	94.16	98.46	52.87	87.41	91.67	55.00
1.56	98.79	99.08	98.72	100.0	78.19	93.16	95.43	72.13
0.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.00

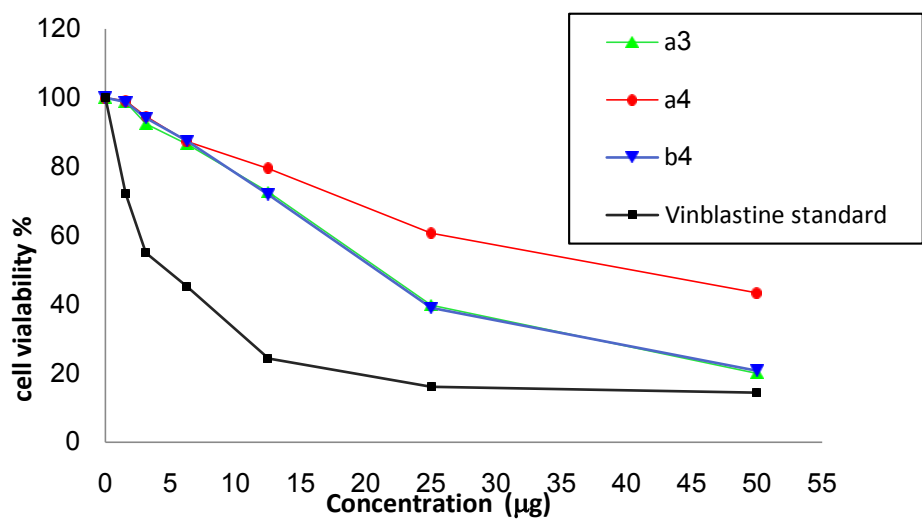


Figure 1: Evaluation of cytotoxicity of compounds (3a, 4a, b).

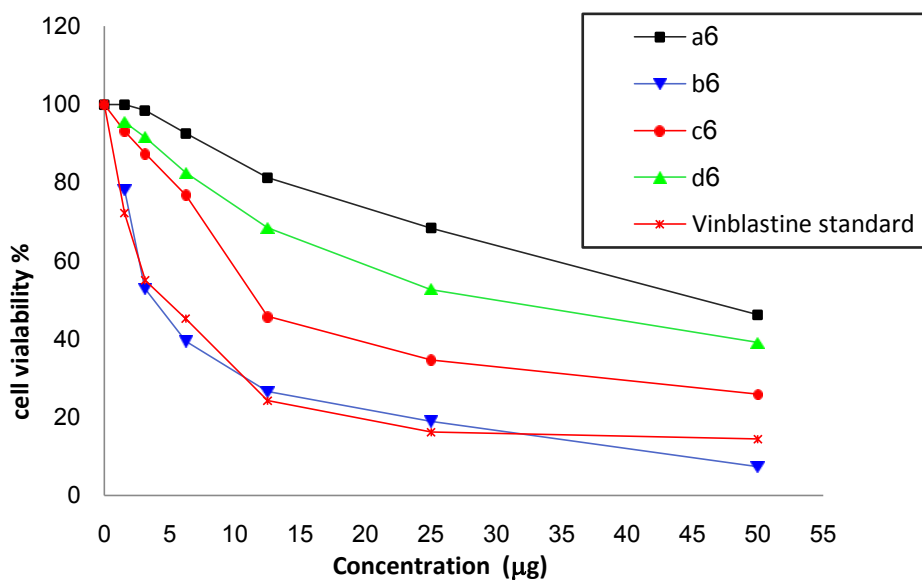
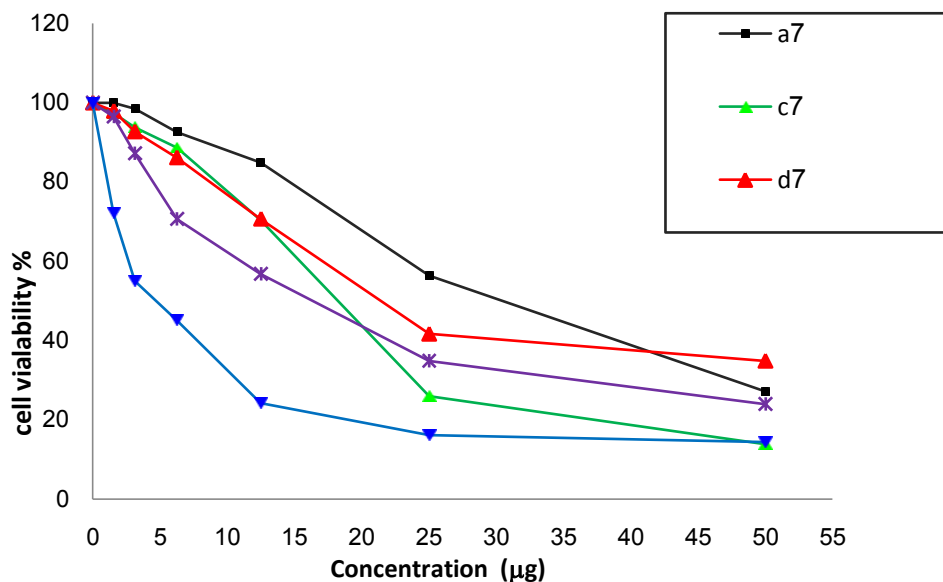


Figure 2: Evaluation of cytotoxicity of compounds (6a-d).

Table 2: Evaluation of cytotoxicity of compounds (7a, 7c, 7d and 9) against HePG2 cell line

Sample conc. (μg)	Viability (%)				
	7a	7c	7d	9	Vinblastine standard
50	27.16	13.83	34.83	23.94	14.38
25	56.41	25.91	41.64	34.82	16.13
12.50	84.92	70.35	70.69	56.76	24.25
6.25	92.65	88.72	86.18	70.62	45.13
3.125	98.46	93.87	92.73	87.19	55.00
1.56	100.00	97.43	97.92	96.56	72.13
0.00	100.00	100.00	100.00	100.0	100.00

**Figure 3: Evaluation of cytotoxicity of compounds (7a-c and 9).**

The results of inhibitory concentration fifty (IC_{50}) data are summarized in table 3.

Table 3: IC_{50} (μg) values of tumor cell lines after 24 hrs continuous exposure to test.

Compd. No.	Tumor cell line (HePG-2)
3a	21.1
4a	40.4
4b	20.8
6a	45.8
6b	3.79
6c	11.7
6d	30.0
7a	30.50
7c	18.2
7d	21.4
9	21.6
Vinblastine standard	16.4
	4.60

IC_{50} : is the concentration that induces 50% growth inhibition compared with untreated cells.
HePG-2= human Hepatocellular carcinoma cell line

In comparison with standard antitumor vinblastine, compound (4a) is found to has more IC_{50} (40.4 μg) than other tested compounds and hence it has lowest activity, while compound (6b) is found to has less IC_{50} value (3.79 μg) than other tested compounds and also less than vinblastine standard itself, hence it has most prominent activity against human Hepatocellular carcinoma (HePG-2).

4. Conclusion

In this paper a new series of 2-thioxo-imidazolidin-4-one derivatives (**3-9**) containing benzylidene amino and phenylethylidene amino groups have been prepared. The structures of these compounds were confirmed by different spectroscopic methods and elemental analysis. The synthesized compounds were evaluated against human Hepatocellular carcinoma (HePG-2), among the tested compounds compound (**6b**) is found to have the lowest IC₅₀ value (3.79 µg), hence it might be potentially useful in the field of cancer treatment and can be suggested as a potent candidate for liver cancer drug.

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