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Synthesis of 2,3,5-trisubstituted-1,2,4-triazine-6-ones as potential antitumor agent

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ABSTRACT

*2,3,5-trisubstituted-1,2,4-triazine-6-ones**(2a,b)**were synthesized by the cyclocondensation of oxazolinonederivatives**(1)**with semicarbazide and thiosemicarbazide. Acylation and condensation of triazine-6-one derivatives**(2a,b)**with acetic anhydride and 3-methoxy-2-hydroxybenzaldehyde yielded the corresponding N-acetyl derivatives**(3a,b)**and N-(3-methoxy-2-hydroxybenzylidene)amino derivatives**(4a,b).**The structure of these synthesized compounds wascharacterized by ^{1}H-NMR, ^{13}C-NMR, Mass, IR and elemental analysis. All the synthesized 1,2,4-triazine-6-ones were tested for their cytotoxicity against HepG-2 cell line. Some of these compounds showed higher cytotoxicity activity than vinblastine standard.*

Keyword:2,3,5-trisubstituted-1,2,4-triazine-6-ones, Antitumor activity

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INTROCUTION

Several derivatives of 1,2,4-triazine-6-one show antimicrobial [1], antibacterial [2], and herbicidal activities [3]. Several investigators found s-triazine nucleus as potential therapeutic agents for diseases due to bacteria, malaria and cancer [4]. 1,2,4-triazine derivatives have been reported to possess a broad spectrum of biological activities, such as antifungal [5,6], anti-HIV [7]and anticancer activities [8].This prompted us to synthesize 2,3,5-trisubstituted of 1,2,4-triazine-6-one and evaluate them for anticancer activities.

MATERIALS AND METHODS

Melting and boiling points were determined in open capillaries using electrothermal digital melting points apparatus and are uncorrected. IR spectra were recorded on Shimadzu (MAU-FOPCU) FT-IR spectrometer using KBR pellets. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker AM 400 FT-NMR spectrometer and chemical shifts were given with respect TMS. Mass spectra were recorded on GC/MS with CI (chemical ionization) and a hewlette-packard MS Engine Thermospray and ionization by electron impact to 70 eV. Microanalysis was conducted using an elemental analyzer 1106.

Synthesis of 4-(p-nitrobenzylidene)-2-phenyl-4H-oxazol-5-one (1)
A mixture of N-(benzoyl)-glycine (0.01 mole), 4-nitrobenzaldehyde (0.01 mole), fused sodium acetate (0.03 mole) and acetic anhydride (0.02 mole) was fused on hot plate for 2-3 min. the reaction mixture was heated on a water-bath for 2 hr, then cooled and poured into water. The solid formed was filtered off, washed with hot water, dried and purified by ethanol to give**(1)**as yellow crystals, yield 76 %, m.p. 237^{o}c. IR (KBR) 1797 (C=O of lactone), 1654 (C=N), 1598,1550 (C=C), 1163,1109 (C-O) Cm^{-1}.^{1}H-NMR (DMSO-d_6):δ7.31-8.59 (m, 10H, Ar-H and H-olefinic) ppm. Anal.Calcd for $C_{16}H_{10}N_2O_4$ (294): C, 65.31; H, 3.40, N, 9.52. Found: C, 65.19; H,3.22; N,9.33.

Synthesis of 5-(p-nitrobenzylidene-3-phenyl-2-aminocarbonyl(aminothiocarnonyl)-1,2,4-triazine-6-ones (2a,b)
A mixture of Oxazolinone derivatives **1** (0.01 mole), semicarbazide hydrochloride and/or thiosemicarbazide (0.01 mole) and fused sodium acetate (0.02 mole) in glacial acetic acid (40 ml) was heated under reflux for 4 hr, the reaction mixture was cooled and poured into water, the resulting solid was filtered off, washed with water, dried and purified by ethanol to give **(2)**.

5-(p-nitrobenzylidene)-3-phenyl-2-aminocarbonyl-1,2,4-triazine-6-ones**(2a)**as pale yellow, yield 71%, m.p. 265^{o}c, b.p. 283^{o}c. IR (KBR): 3419, 3217(NH_2), 3267 (NH), 1730, 1714 (C=O), 1637 (C=N), 1597,1516 (C=C), 1107 (C-O) cm^{-1}.^{1}H-NMR (DMSO-d_6):δ6.50 (br.s, 1H, OH), 7.39-8.59 (m, 12H, Ar-H, NH_2 and H-olefinic), 9.05 (br.s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6):δ169.45, 165.32 (C=O), 148.25 (C-O), 147.9 (C=N), 140.87, 140.07, 139.49, 139.49, 136.71, 134.76, 133.69, 133.39, 133.29, 133.04, 129.89, 129.40, 129.30, 129.15, 128.87, 127.89, 127.44, 125.55, 125.28, 124.65, 124.26 (C-aromatic and triazine ring) ppm. MS: m/z (%) = 352(M^++1, 1.7), 351 (M^+, 11.70), 350 (M^+-1, 56.50), 335(2.4), 334(2.20), 320 (1.2), 319 (4.70), 309 (18.7), 308 (100), 294 (11.10), 293 (34.50), 291 (7.60), 263 (1.3), 262 (3.3), 261 (3.7), 247 (3.40),246 (4.6), 245 (2.20), 236 (1.3), 233 (1.40), 232 (1.30), 218 (1.20), 217 (2.10), 205 (1.80), 204 (3.40), 203 (3.1), 176 (3.10), 175 (6.80), 162 (5.90), 161 (15.60), 151 (1.6), 150 (1.90).149 (7.90), 147 (3.60), 132 (2.20), 131(2.30), 129 (4.00), 120 (5.80), 119 (48.0), 118 (48), 117 (5.10), 106 (4.10), 105 (53.50), 104 (42.0), 103 (12.60), 101 (11.70), 91 (1.60), 90 (2.2) ,89 (8.80), 85 (2.40), 83 (5.10), 78 (2.70), 77 (32.8), 76 (8.00), 75 (10.00), 52 (1.1), 51 (6.6). Anal.Calcd for $C_{17}H_{13}N_5O_4$ (351): C, 58.12; H, 3.70; N, 19.94. Found: C, 58.03; H, 3.58; N, 19.78.

5- (p-nitrobenzylidene)-3-phenyl-2-aminothiocarbonyl-1,2,4-triazine-6-one **(2b)** as yellow crystals, yield 73%, m.p. 220^{o}c, b.p. 230^{o}c.IR (KBR): 3422, 3163 (NH_2), 3285 (NH), 1735 (C=O), 1637 (C=N),1600,1516 (C=C),1492 (C=S), 1101 (C-O) Cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ7.33-8.529 (m, 12 H, AR-H, NH_2 and H-olefinic), 10.72 (S, 1H, NH), 11.71 (S, 1H, OH) ppm.^{13}C-NMR (DMSO-d_6):δ184.97, 182.43 (C=S), 178.96 (C=O), 168.59 (C=O),161.50 (C-O), 148.03 (C=N),141.19, 140.80, 140.01, 139.33, 133.54, 133.35, 132.92, 132,62, 131.14, 130.29, 129.95, 129.43, 129.11, 129.02, 128.93, 128.30, 128.16, 124.49, 124.91, 124.30 (C-aromatic and C-triazine ring) ppm. MS: m/z (%) = 368 (M^++1,6.50), 366 (M^+-1,1.00), 340 (1.10), 339 (1.20), 324(1.100), 319 (1.30), 296 (1.4), 295 (7.00),294 (21.80), 280(1.20), 279 (1.0), 278 (1.20), 235 (1.10), 234 (1.20), 219 (1.30), 218 (1.80), 205 (1.40), 204 (1.20),178 (1.10), 177 (1.10), 176 (1.00), 174 (1.40), 172 (1.80), 164 (1.40), 163 (2.8),162 (3.90), 161 (2.20), 159 (1.30), 157 (1.30), 157 (1.30), 152 (1.10), 150 (2.40), 149 (1.70), 144 (2.00), 143 (1.40), 133 (1.30), 132 (3.10), 131 (1.90), 130 (2.30), 129 (2.40), 128 (2.00), 122 (6.40), 121 (14.60), 119 (3.40), 118 (3.50), 116 (6.30), 115 (7.10), 114 (3.60), 107 (2.70), 106 (27.60), 105 (100), 102 (5.60), 101 (2.40), 91 (4.10), 90 (4.00), 89 (10.10), 88 (4.60), 87 (2.20), 78 (13.10), 77 (100), 75 (8.10), 74 (5.50), 65 (3.00),64 (2.60), 63 (7.50), 62 (4.50), 52 (4.10), 51 (30.80). Anal.Calcd for$C_{17}H_{13}N_5O_3S$ (367): C, 55.58; H, 3.54; N, 19.07. Found: C, 55.39; H, 3.33; N, 19.98.

Synthesis of 5-(p-nitrobenzylidene)-3-phenyl-2-substituted-1,3,4-triazine-6-ones (3)
A solution of **2** (0.01 mole) in mixture of acetic anhydride and acetic acid (15:15 ml) was heated under reflux for 2 hr, then cooled and poured into ice-water. The resulting product was filtered off, washed with water, dried and purified by recrystallization from benzene to give **(3).**

5-(p-nitrobenzylidene)-3-phenyl-2-(acetylamino)carbonyl-1,2,4-triazine-6-one **(3a)** as pale yellow crystals, yield 63%, m.p. 190^{o}c, b.p. 200^{o}c. IR (KBR): 3103 (NH), 1733-1715 (br.C=O), 1639 (C=N), 1558,1517 (C=C), 1168,1105 (C-O) Cm^{-1}. ^{1}H-NMR (DMSO-d_6):δ2.50 (S, 3H, $COCH_3$), 7.46-8.61 (m, 10 H, Ar-H and H-olefinic) ppm.^{13}C-NMR (DMSO-d_6):δ170.82, 168.50 (C=O), 160.86 (C=O), 148.33 (C=N), 140.18, 137.84, 136.68, 134.75, 133.96, 133.73, 133.38, 129.90, 128.47, 127.45, 126.70, 125.26, 127.45, 124.33 (C-aromatic), 24.97 ($COCH_3$) ppm. MS: m/z (%) = 394 (M^++1, 9.90), 393 (M^+, 48.70), 392 (M^+-1, 61.20), 351 (25.00), 350 (62.10), 334 (2.20), 333 (1.80), 310 (2.80), 309 (21.90), 308 (97.20), 294 (1.80), 293 (2.60), 292 (3.80), 291 (7.20), 279 (1.10), 278 (1.20), 250 (1.90), 246 (1.10), 234 (1.10), 233 (1.90), 232 (1.30), 219 (22.00), 205 (2.30), 204 (4.90), 203 (3.70), 177 (1.60), 176 (3.10), 175 (2.80), 162 (3.20), 161 (24.50), 147 (3.80), 145 (2.00), 144 (1.20), 131 (1.30), 130 (1.70), 129 (2.70), 128(1.60), 120 (9.10), 119 (100), 117 (5.20), 116 (2.00), 115 (3.20), 114 (2.80), 106 (3.20), 105 (43.00), 104 (28.90), 103 (12.20), 101 (14.30), 92 (1.70), 91 (1.50), 90 (2.60), 89 (12.00), 78 (2.60), 77 (31.40), 76 (8.60), 75 (13.00), 64 (1.10), 63 (4.80), 52 (1.10), 51 (6.90). Anal.Calcd. For $C_{19}H_{15}N_5O_5$ (393): C, 58.01; H, 3.82; N, 17.81. Found: C, 57.97; H, 3.71; N, 17.67.

5-(p-nitrobenzylidene)-2-phenyl-2-(acetylamino)-thiocarbonyl-1,2,4-triazine-6-one **(3b)** as pale yellow crystals, yield 61%, m.p.165^{o}c, b.p. 230^{o}c. IR (KBR): 3102 (NH), 1735-1725 (br.C=O), 1641 (C=N), 1595,1558 (C=C), 1448 (C=S) Cm^{-1}.^{1}H-NMR (DMSO-d_6):δ2.50 (S, 3H, $COCH_3$),7.53-8.62 (m, 10H, Ar-H and H-olefinic) ppm. ^{13}C-NMR (DMSO-d_6): δ170.82 (C=S), 168.50, 160.87 (C=O), 148.39 (C=N), 140.19, 137.84, 136.71, 134.76, 133.73, 133.39, 129.90, 128.87, 128.47, 127.44, 126.70, 125.28, 124.36, 124.34 (C-aromatic), 24.97 ($COCH_3$) ppm. MS: m/z (%) = 410 (M^++1, 1.30), 409 (M^+,6.30), 367 (16.20), 366 (11.20), 340 (1.13), 339 (1.10), 324 (1.40), 319 (1.30), 296

(1.35). 295 (6.30), 294 (20.10), 280 (1.30), 279 (1.10), 278 (1.10), 235 (1.20), 234 (1.30), 219 (1.35), 218 (1.90), 205 (1.40), 204 (1.10), 178 (1.30), 177 (1.30), 174 (1.43), 172 (1.70), 164 (1.40), 163 (2.73), 162 (3.80), 161 (2.30), 159 (1.20), 157 (1.20), 152 (1.20), 150 (2.50), 149 (1.60), 144 (2.10), 143 (1.50), 133 (1.40), 132 (3.01), 131 (1.80), 130 (2.31), 122 (6.30), 121 (14.80), 119 (3.40), 118 (3.60), 116 (6.40), 115 (6.20), 114 (3.50), 107 (2.70), 106 (28.60), 105 (100), 102 (5.60), 101 (3.40), 91 (4.00), 90 (4.10), 89 (11.10), 88 (4.50), 87 (2.30), 78 (12.10), 77 (100), 75 (9.10), 74 (5.10), 65 (2.20), 64 (2.70), 63 (7.70), 62 (4.40), 52 (5.10), 51 (6.30). Anal.Calcd. For $C_{19}H_{15}N_5O_4S$ (409): C, 55.74; H, 3.67; N, 17.11. Found: C, 55.55; H, 3.52; N, 17.02.

Synthesis of 5-(p-nitrobenzylidene)-3-phenyl-2-substitituedcarbonyl or (thiocarbonyl)-1,2,4-triazine-6-ones (4)

A mixture of **2** (0.01 mole) and 3-methoxy-2-hydroxy-benzaldehyde (0.01 mole) in glacial acetic acid (50 ml) was heated under reflux for 4 hr, then cooled and poured into ice-water. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give **(4).**

5-(p-nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-hydroxybenzylidene)amino]carbonyl-1,2,4-triazine-6-one **(4a)** as yellow crystals, yield 71%, m.p. 210^{o}c, b.p. 230^{o}c.IR (KBR): 3421-2841 (br.OH), 3215 (NH), 1721,1697 (C=O), 1651, 1622 (C=N), 1598, 1517 (C=C), 1249 (C-O) Cm^{-1}. ^{1}H-NMR (DMSO-d_6):δ3.89 (S, 3H, OCH_3), 6.89- 8.60 (m, 13H, Ar-H and olefinic H), 8.98 (S, 1H, CH=N), 10.11 (S, 1H, NH), 10.89 (S, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6):δ166.41, 163.24 (C=O), 148.96, 148.43, 148.27 (C-O), 147.40 (C=N), 141.24, 140.06, 136.71, 134.76, 133.71, 133.39, 132.47, 131.59, 131.01, 129.88, 129.73, 129.73, 129.30, 128.97, 128.41, 128.26, 127.89, 127.44, 125.28, 124.36, 124.08, 119.76, 118.80, 115.75 (C-aromatic), 56.36 (OCH_3)ppm.MS: m/z (%): 486 (M^++1, 1.30), 485 (M^+, 6.20), 444 (2.90), 443 (13.40), 442 (40.90), 413 (1.20), 412 (2.20), 352 (3.60), 351 (11.20), 350 (5.60), 321 (1.10), 320 (2.60), 319 (2.20), 313 (1.4), 312 (1.60), 309 (19.50), 308 (95.70), 294 (26.60), 293 (74.90), 292 (14.90), 278 (4.20), 277 (8.10), 276 (37.80), 263 (3.20), 262 (3.90), 261 (2.90), 249 (1,70), 248 (7.60), 247 (35.50), 246 (28.20), 239 (1.90), 236 (1.40), 234 (1.20), 233 (1.70), 221 (2.20), 220 (1.30), 218 (1.70), 217 (1.30), 205 (2.30), 204 (4.40), 203 (4.00), 191 (3.50), 190 (10.00), 189 (2.50), 178 (1.80), 177 (2.10), 176 (3.90), 175 (5.00), 165 (1.10), 164 (1.60), 163 (5.10), 162 (34.70), 153 (1.00), 152 (2.10), 151 (5.20), 150 (17.00), 149 (11.80), 147 (5.40), 145 (8.30), 144 (10.90), 143 (2.60), 121 (5.50), 120 (11.00), 119 (100), 118 (7.70), 116 (8.30), 115 (7.30), 114 (4.50), 108 (14.60), 107 (8.10), 106 (14.00), 105 (94.60), 104 (57.60), 103 (24.40), 101 (13.00), 89 (14.40), 88 (5.80), 78 (10.40), 77 (88.70), 76 (15.90), 75 (12.40), 65 (4.70), 64 (4.10), 63 (9.30), 52 (7.90), 51 (16.20). Anal.Calcd for $C_{25}H_{19}N_5O_6$ (485): C, 61.86; H, 3.92; N, 14.43. Found: C, 61.67; H, 3.73; N, 14.24.

5-(p-nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-hydroxybenzylidene)amino]-thiocarbonyl-1,2,4-triazine-6-one **(4b)** as yellow crystals, yield 73%, m.p. 205^{o}c, b.p.235^{o}c. IR (KBR): 3459-2890 (br.OH),3196 (NH), 1720-1705 (C=O), 1633 (C=N), 1597, 1556 (C=C), 1446 (C=S), 1251,1165 (C-O) Cm^{-1}. ^{1}H-NMR (DMSO-d_6):δ 3.88 (S, 3H, OCH_3), 6.88-8.56 (m, 13H, Ar-H and H-olefinic), 8.97 (S, 1H, CH=N), 10.13 (S, 1H, NH), 11.71 (S, 1H, OH) ppm.^{13}C-NMR (DMSO-d_6): δ 192.49, 182.45 (C=S), 168.60, 163.26 (C=O), 151.17, 148.80 (C-O), 147.96, 141.23 (C=N), 140.80, 140.02, 139.29, 136.63, 134.74, 133.54, 132.91, 132.47, 131.18, 130.44, 129.85, 129.77, 129.42, 129.11, 128.97, 128.85, 128.26, 127.49, 125.53, 125.22, 124.65, 124.23, 122.93, 122.53, 120.62, 119.75, 118.78, 118.03, (C-aromatic), 56.45 (OCH_3) ppm. Anal.Calcd. For $C_{25}H_{19}N_5O_5S$ (501): C, 59.88; H, 3.79; N, 13.97. Found: C, 59.65; H, 3.63; N, 13.78.

Synthesis of 5-(p-nitrobenzylidene)-3-phenyl-2-substituted-carbonyl(thiocarbonyl)-1-acetyl-1,2,4-triazine-6-ones (5a,b)

A solution of **4** (0.01 mole) in acetic anhydride (30 ml) was heated under reflux for 2 hr, then cooled and poured into ice-water. The solid formed was filtered off, washed with water, dried and purified by recrystallization from benzene to give **(5).**

5-(p-nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-acetoxy-benzylidene)amino]carbonyl-1-acetyl-1,2,4-triazine-6-one **(5a)** as pale yellow crystals, yield 63%, m.p. 187^{o}c, b.p. 230^{o}c.^{1}H-NMR (DMSO-d_6):δ 2.45 (S, 3H, $COCH_3$), 2.50 (S, 3H, $COCH_3$), 3.82 (S, 3H, OCH_3), 7.10-8.59 (m, 13H, Ar-H and H-olefinic), 8.64 (S, 1H, CH=N) ppm. ^{13}C-NMR (DMSO-d_6):δ 169.63, 168.50, 166.89, 165.32 (C=O), 157.20, 151.11 (C-O), 148.34, 140.05 (C=N), 139.80, 137.86, 136.70, 134.76, 133.97, 133.38, 130.47, 129.88, 129.03, 128.46, 127.44, 127.26, 125.27, 124.35, 119.65, 115.95 (C-aromatic), 56.60 (OCH_3), 24.96, 20.77 (2 x $COCH_3$) ppm. MS: m/z (%)=570 (M^++1, 1.20), 569 (M^+, 2.20), 528 (3.00), 527 (9.00), 486 (2.70), 485(10.50), 484 (18.90), 444 (3.40), 443 (14.40), 442 (44.20), 426 (1.10), 425 (1.40), 413 (2.60), 411 (1.10), 397 (1.90), 396 (1.10), 353 (1.10), 352 (1.30), 351 (4.40), 350 (13.20), 343 (1.10), 342 (3.90), 321 (1.60), 320 (4.90), 319 (2.60), 309 (6.00), 308 (27.30), 300 (4.40), 295 (6.50), 294 (32.90), 293 (100), 291 (4.00), 284 (1.10), 283 (4.10), 278 (4.60), 277 (11.20), 276 (50.10), 264 (2.10), 263 (2.50), 262 (2.40), 261 (2.10), 250 (3.70), 249 (2.40), 248 (10.10), 247 (44.30), 246 (30.10), 236 (1.90), 235 (1.50), 221 (2.10), 220 (3.10), 218 (1.80), 204 (4.00), 203 (4.00), 191 (4.00), 190 (12.40), 189 (2.80), 178 (2.30), 177 (2.80), 176

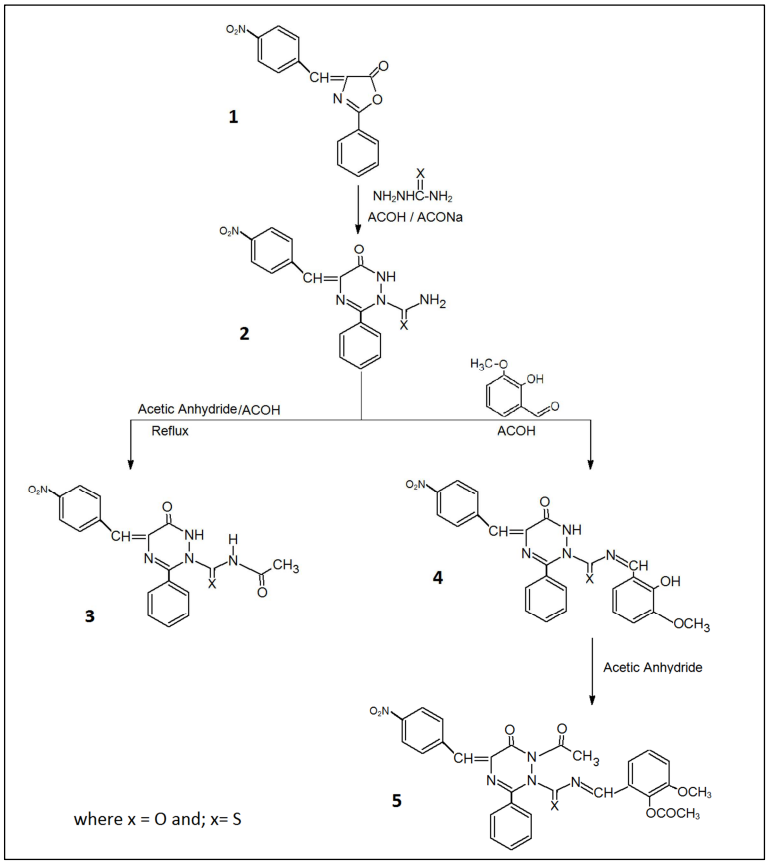
(3.50), 175 (3.40), 163 (6.60), 162 (38.70), 161 (6.90), 152 (3.40), 151 (10.00), 150 (21.90), 149 (14.50), 148 (4.90), 145 (3.10), 144 (10.70), 136 (5.30), 135 (37.50), 134 (5.50), 132 (11.10), 131 (4.20), 121 (3.80), 120 (6.10), 119 (20.70), 116 (7.40), 108 (10.00), 107 (11.70), 106 (13.50), 105 (40.40), 104 (41.00), 103 (16.40), 93 (5.70), 92 (6.40), 91 (5.20), 89 (9.90), 78 (6.50), 77 (27.20), 76 (8.60), 65 (5.60), 64 (3.40), 63 (5.70), 52 (3.60), 51 (8.60). Anal.Calcd for $C_{29}H_{23}N_5O_8$ (569): C, 61.16; H, 4.04; N, 12.30. Found: C, 61.02; H, 3.98; N, 12.12.

5-(p-nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-acetoxybenzylidene)amino]-thiocarbonyl-1-acetyl-1,2,4-triazine-6-one **(5b)** as pale yellow, yield 61%, m.p. 171^{o}c, b.p. 235^{o}c. IR (KBR): 1795,1735 (C=O), 1641 (C=N), 1597,1558 (C=C), 1448 (C=S), 1165,1107 (C-O) Cm^{-1}.^{1}H-NMR (DMSO-d_6):δ 2.45 (S, 3H, $COCH_3$), 2.50 (S, 3H, $COCH_3$), 3.83 (N, 3H, OCH_3), 7.36-8.58 (m, 13H, Ar-H and H-olefinic), 8.61 (S, 1H, CH=N) ppm. ^{13}C-NMR (DMSO-d_6):δ170.82 (C=S), 168.50, 166.86, 165.30 (C=O), 160.85 (C-O), 148.32, 148.24 (C=N), 140.16, 140.02, 137.83, 136.65, 134.74, 133.96, 133.73, 133.67, 133.37, 129.86, 128.85, 128.46, 127.45, 127.28, 126.69, 125.24, 124.32 (C-aromatic), 56.40 (OCH_3), 24.96, 20.76 (2 x $COCH_3$) ppm. Anal.Calcd for $C_{29}H_{23}N_5O_7S$ (585): C, 59.49; H, 3.93; N, 11.96. Found: C, 59.27; H, 3.73; N, 11.79.

RESULTS AND DISSCUTION

a. Chemistry

4-(p-nitrobenzylidene)-2-phenyl-4H-oxazol-5-one **(1)**was synthesized from N-(benzoyl)-glycine and 4-nitrobenzaldehyde in the presence of acetic anhydride and fused sodium acetate according to published literature procedure [9].The cyclocondensation [10] of oxazolinone**(1)**, with semicarbazide and thiosemicarbazide in glacial acetic acid in presence of fused sodium acetate to yield the corresponding 5-(p-nitrobenzylidene)-3-phenyl-2-(aminocarbonyl) and/or (aminothiocarbonyl)-1,2,4-triazine-6-one**(2a,b)**. Acylation of 2-(aminocarbonyl)- and/or -(aminothiocarbonyl)-1,2,4-triazine-6-one derivatives**(2a,b)**with acetic anhydride and acetic acid under reflux afforded the corresponding 5-(p-nitrobenzylidene)-3-phenyl-2-(acetylamino)carbonyl- and/or (acetylamino)thiocarbonyl-1,2,4- triazine-6-ones**(3a,b)**.



Scheme1

Condensation of 2-(aminocarbonyl)-and/or (aminothiocarbonyl)-1,2,4-triazine-6-one derivatives**(2a,b)** with 3-methoxy-2-hydroxybenzaldehyde using glacial acetic acid as reaction solvent to afford the corresponding 5-(p-

nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-hydroxy benzylidene)amino]-carbonyl (thiocarbonyl)-1,2,4-triazine-6-ones**(4a,b)**.Acetylation of compound 4 with acetic anhydride under boiling to give 5-(p-nitrobenzylidene)-3-phenyl-2-[(3-methoxy-2-acetoxybenzylidene)amino]-carbonyl (thiocarbonyl)-1-acetyl-1,2,4-triazine-6-ones**(5a,b)**

a. Mass spectra of some 1,2,4-triazine-6-ones derivatives

The mass spectral fragmentation patterns of some prepared 1,2,4-triazine-6-ones derivatives such as 2-aminocarbonyl(aminothiocarbonyl)-3-phenyl-5-(p-nitrobenzylidene)-1,2,4-triazine-6-ones**(2a, b)**, N-acetyl derivatives**(3a,b)**and 2-[(3-methoxy-2-hydroxybenzylidene)amino]-carbonyl-3-phenyl-5-(p-nitrobenzylidene)-1,2,4-triazine-6-one**(4a)**are investigated in order to elucidate the structure of the synthesized compounds.

Compounds 2a and 2b

The mass spectra of compounds**2a**and**2b**showed intense molecular ion peaks at m/z 351 and 367, consistent with molecular formula $C_{17}H_{13}N_5O_4$and $C_{17}H_{13}N_5O_3S$, respectively. The molecular ion of (m/z 351,**Figure 1**)and m/z 367 for compounds**2a,b**fragmented further along different various pathways as illustrated in **chart 1 and 2.**

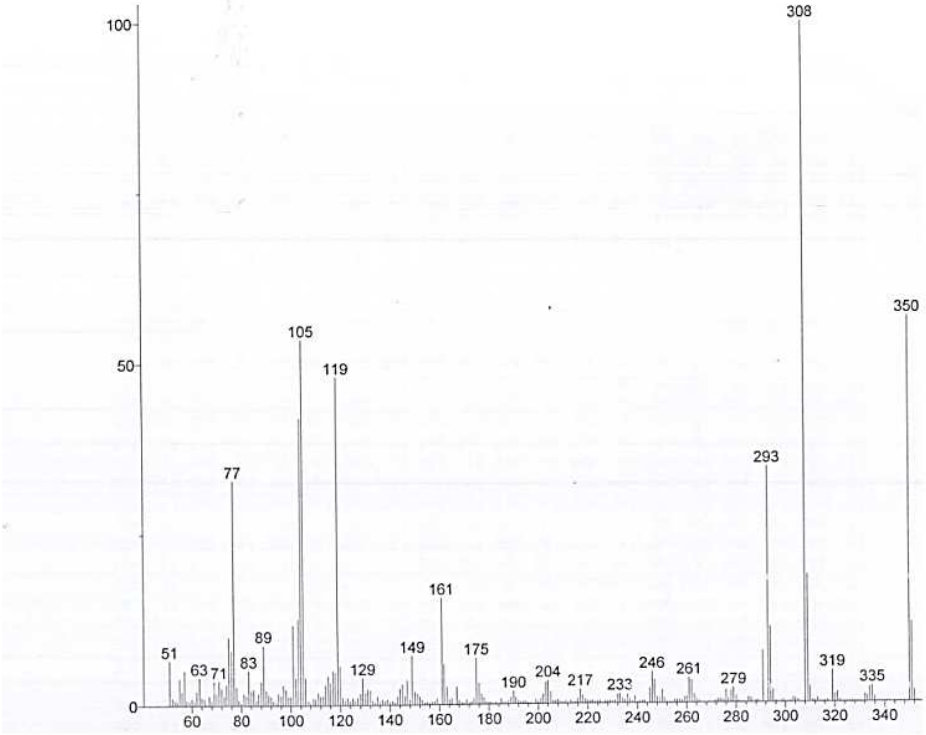


Figure 1: Mass spectrum of compound 2a

Compound 3a

The molecular ion of compound**3a**(m/z 393,**Figure 2**) had fragmented to give the fragment of m/z 351, corresponding to the molecular ion of compound**2a**by losing ketene molecule (CH_2=C=O).The fragment of m/z 351 was broken via pathway in the same fragmented processes which was observed for compound**2a (Chart 1 and 2)**.

Compound 4a

The mass spectra of compound**4a(Figure 3)**is fully consistent with assigned structure. Intense molecular ion peaks was observed at m/z 485. The molecular ion peak at m/z 485 fragmented further and involved different pathways as illustrated in**chart 3 and 4**.

The mass spectra of the prepared 1,2,4-triazine-6-one derivatives**(2,3,4**and **5)**showed different common peaks at m/z 308, 293, 119, 105 and m/z 77 for all these compounds.

The electron impact ionization mass spectra of compounds **2a**and**3a** show a weak molecular ion peak and base peaks at m/z 308 and m/z 119, resulting from a cleavage fragmentation. The compound**4a**gives a characteristic fragmentation Pattern with a very stable fragment at m/z 293.

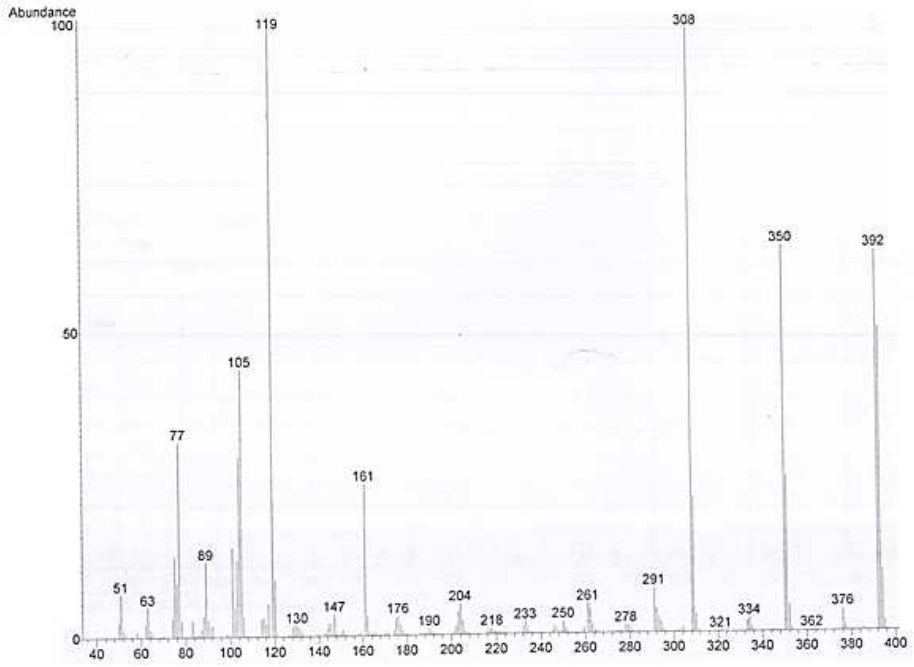


Figure 2: Mass spectrum of compound 3a

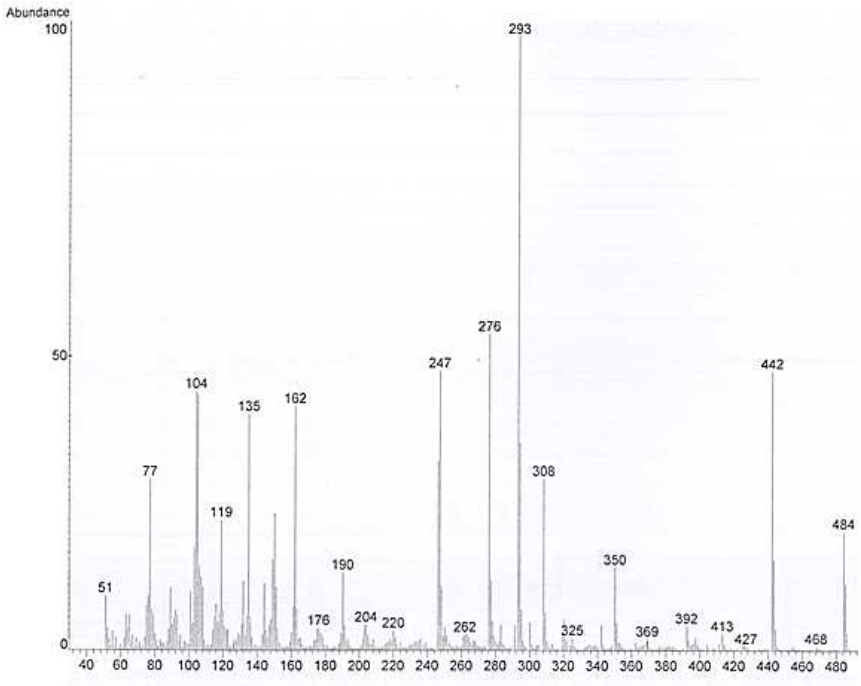


Figure 3: Mass spectrum of compound 4a

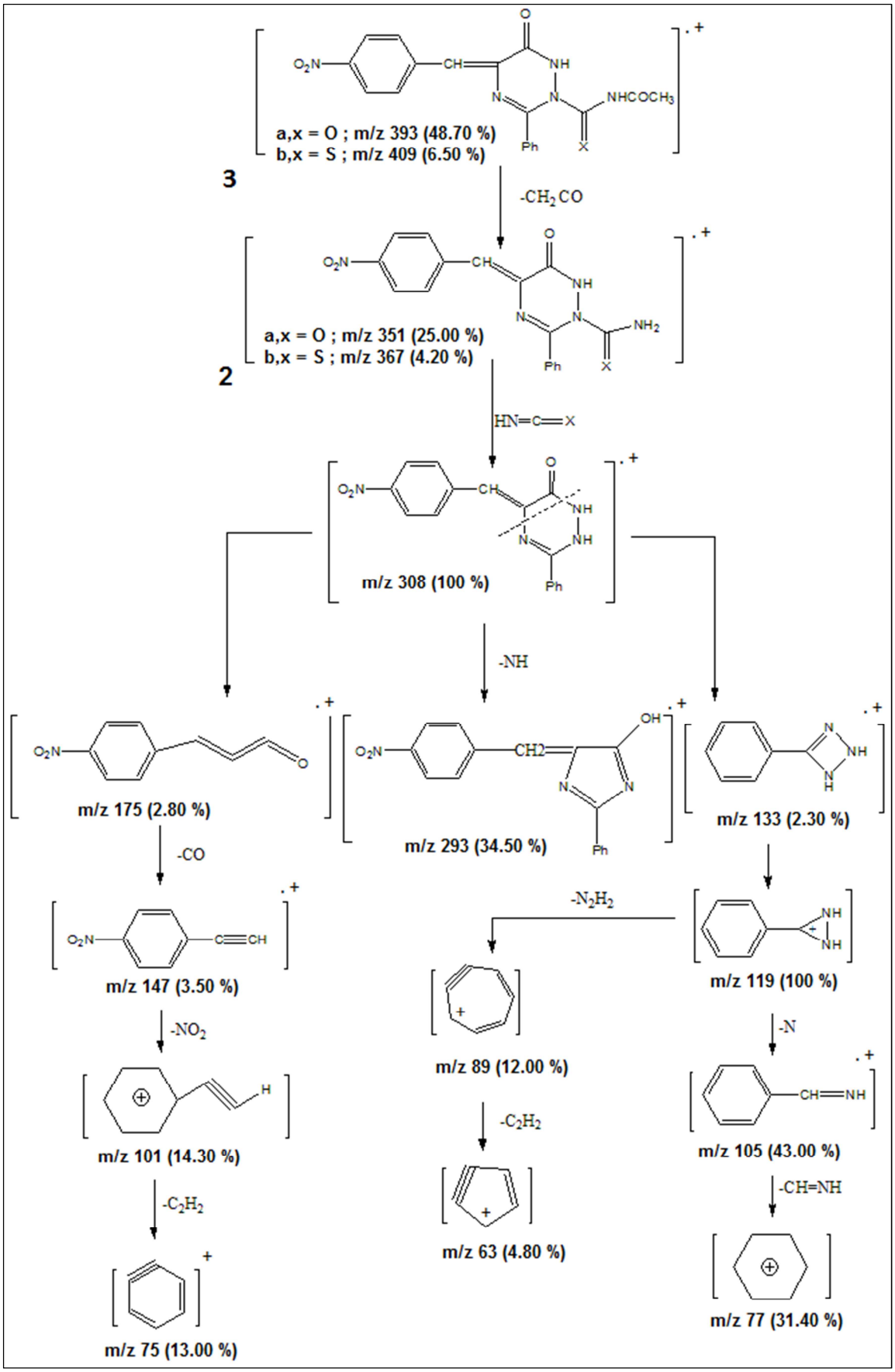


Chart 1: Main fragmentations pathways of compounds 2 and 3

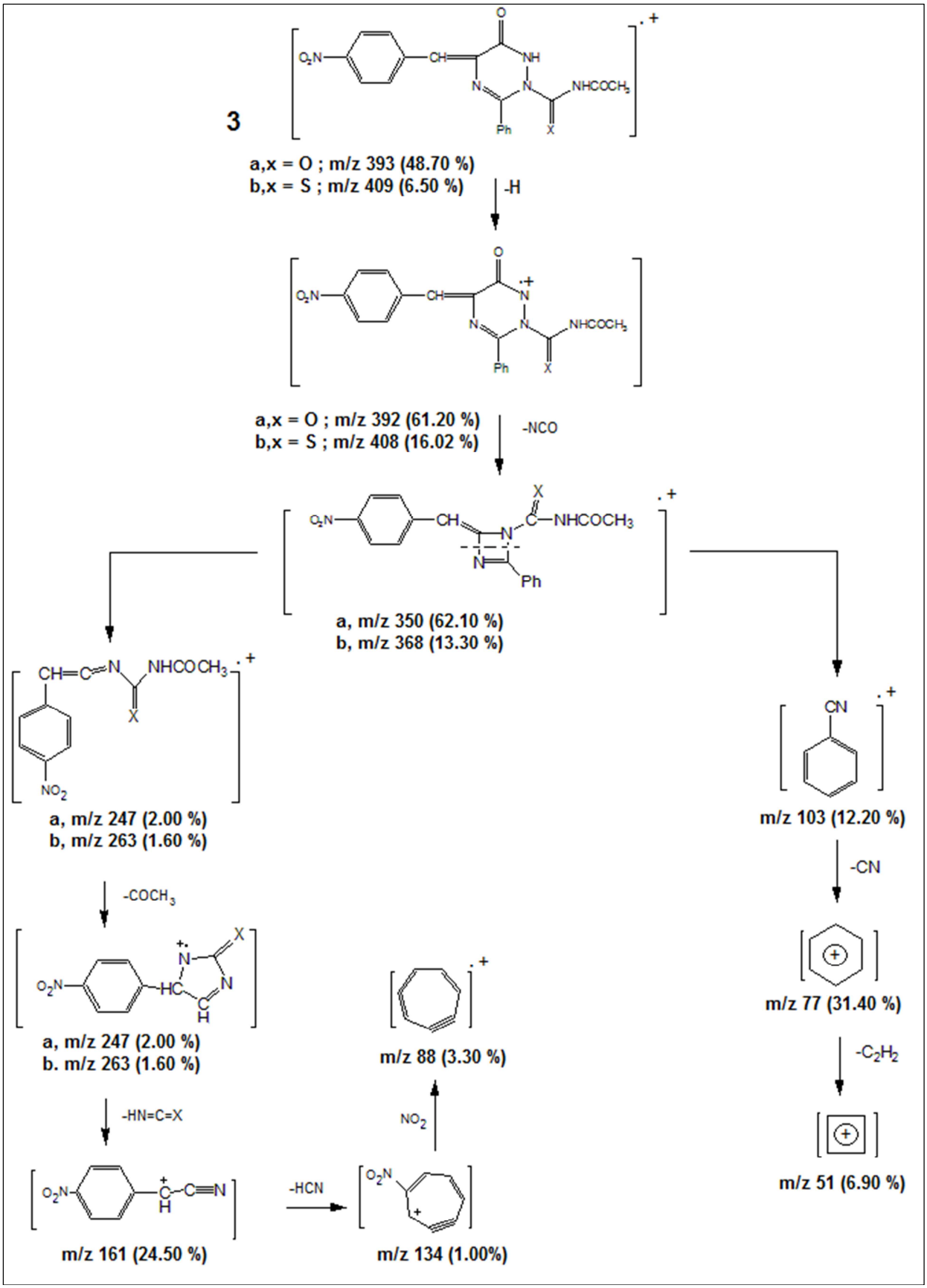


Chart 2: main fragmentations pathways of compounds 2 and 3

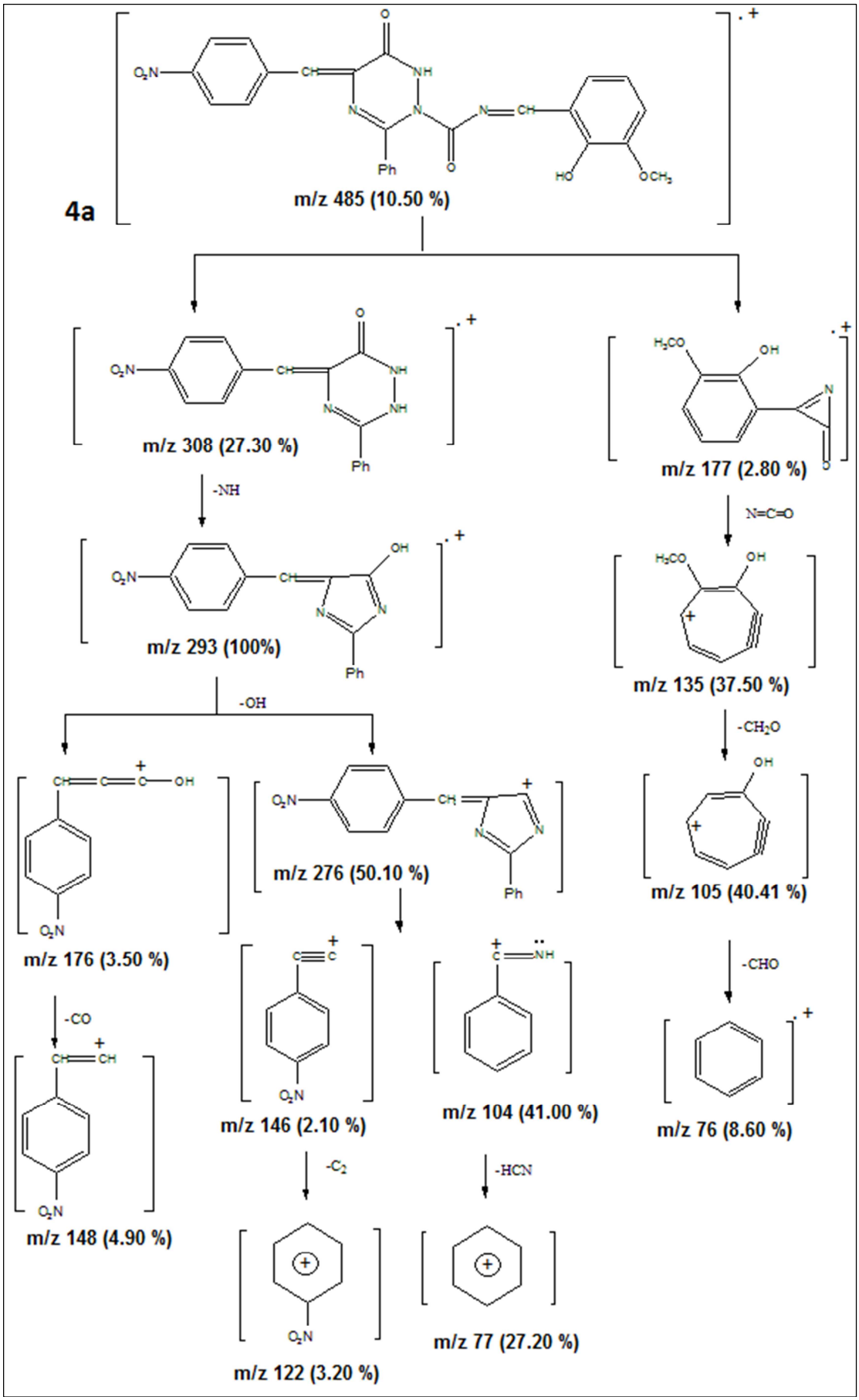


Chart 3: Main fragmentation pathways of compound 4a

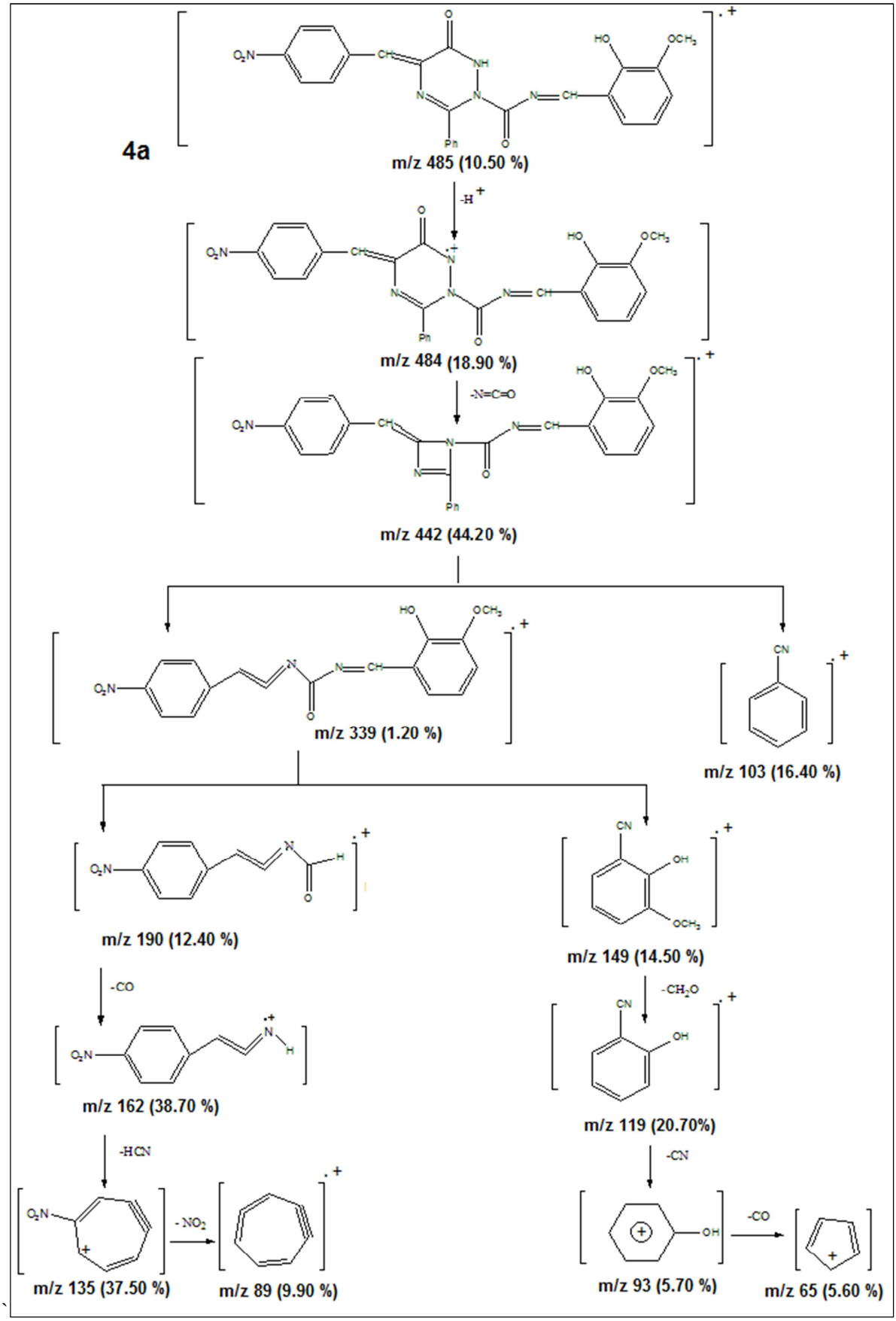


Chart 4: Main fragmentation pathways of compound 4a

b. Anticancer activity

All the synthesized 1,2,4-triazine-6-ones derivatives**2a,b, 3a,b, 4b**and**5b**were evaluated for their cytotoxicity against hepatocellular carcinoma cells (HepG-2 cell line) by mosamann and vijayan et al assay [11,12]. The 50% inhibitory concentration (IC_{50}) of HepG-2 cell line was calculated from **table 1**and **figure 1** and **2**.

Table (1): Evaluation of cytotoxicity of 1,2,4-triazine-6-ones derivatives against HepG-2 cell line

Sample Concentration (µg)	Viability %						
	2a	2b	3a	3b	4b	5b	Vinblastine standard
50	18.74	7.45	8.21	9.36	35.89	6.39	7.82
25	29.16	15.39	19.83	17.69	49.08	12.81	15.18
12.5	41.53	22.76	26.08	23.05	61.75	20.42	29.60
6.25	62.97	31.49	32.64	32.48	85.63	27.58	48.75
3.125	79.41	40.75	41.95	47.56	93.16	38.73	60.35
1.56	88.15	59.27	58.02	63.92	98.16	56.84	76.24
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The IC_{50} values of 1,2,4-triazine-6-one derivatives are cited in **table 2.**

Table (2): IC_{50} (µg) of 1,2,4-triazine-6-ones derivatives after 48h continuous exposure of tumor cell line

Compound No.	2a	2b	3a	3b	4b	5b	Vinblastine Standard
HepG-2 Cell line	10.0	2.34	2.34	2.89	24.10	2.15	4.60

The IC_{50}values are the concentration that induces 50% growth inhibition compared with untreated control cells.
HepG-2: Human hepatocellular carcinoma cell line.

Compounds **2b** (IC_{50} 2.34 µg), **3a** (IC_{50} 2.34 µg), **3b** (IC_{50} 2.89 µg) and **5b** (IC_{50} 2.15 µg) were found to exhibit very good cytotoxic activity against HepG-2 cell line. Compounds **2a** (IC_{50} 10.0 µg), and **4b** (IC_{50} 24.10 µg), showed good cytotoxicity activity.

In comparison with standard antitumor drug vinblastine, compounds **2b, 3a,b** and **5b** were found to be more active than standard drug, while compounds **2a**, and **4b** were observed to be less active than standard drug vinblastine against HepG-2.

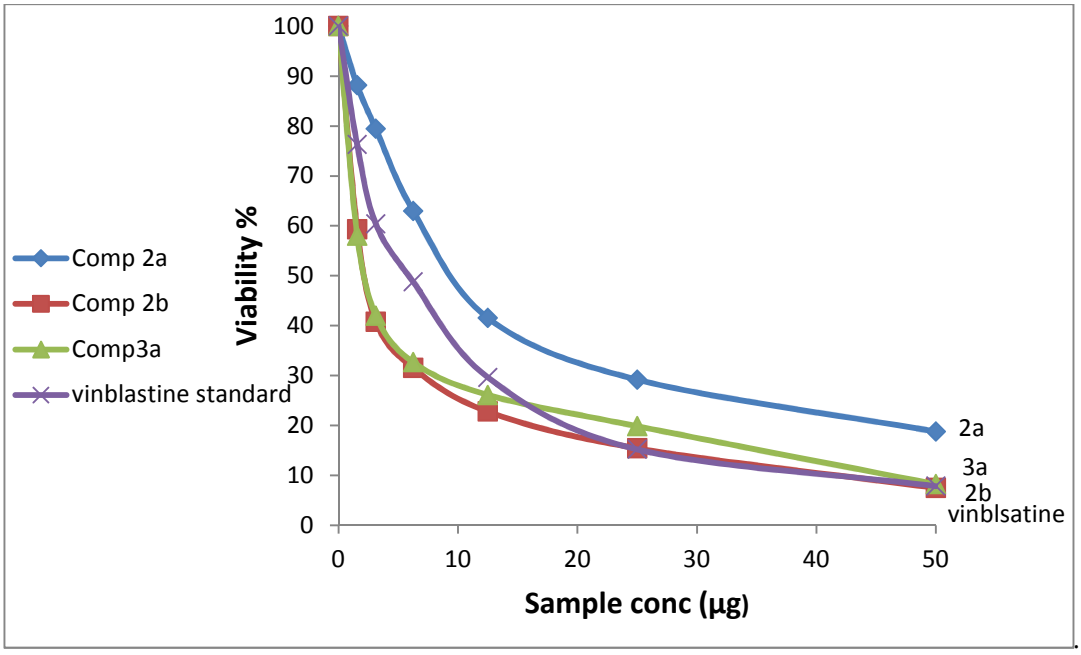


Figure 4

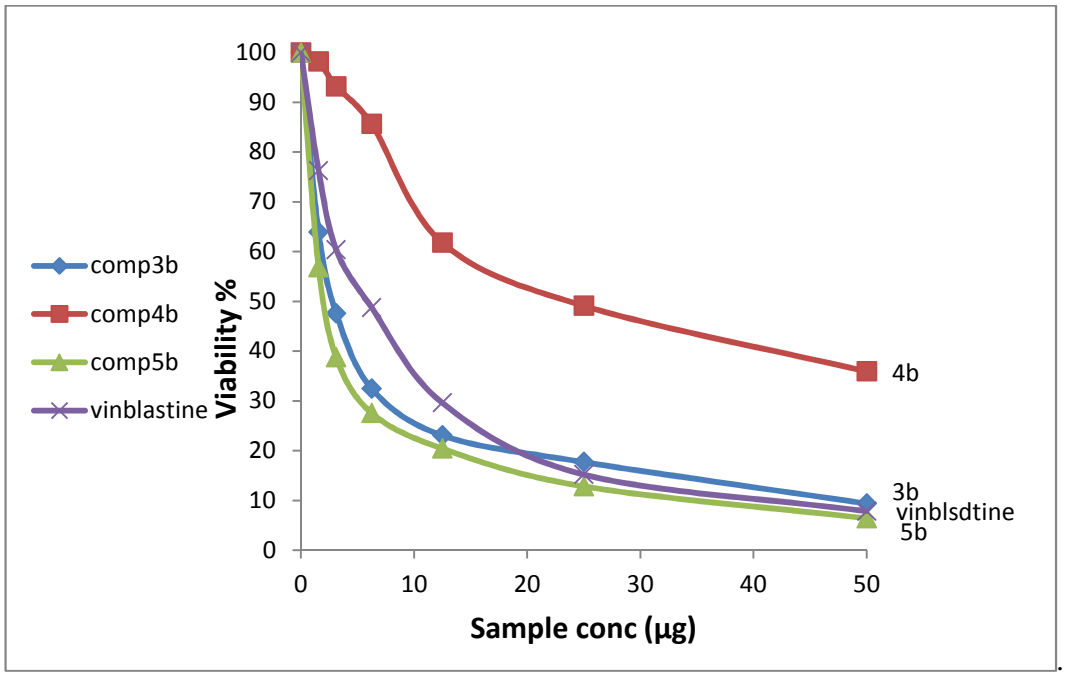


Figure 5

CONCLUSION

1- Synthesis of 2,3,5-trisubstituted-1,2,4-triazine-6-ones.The structures of these compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis.
2- The triazine-6-one derivatives showed better antitumor activities.
3- Furthermore the substitution with acetyl group in 1,2,4-triazine-6-one derivatives showed good activities against HepG-2 cell line.
4- 1,2,4-triazine-6-ones and its derivatives were found to play an important role in medical chemistry as anticancer activity.

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