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New Potential Antitumor Nitrogen Heterocycles: Synthesis and Cytotoxic Evaluation

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ABSTRACT

*A new 2-(p-nitrobenzylidene)-4-phenyl-5-oxo(thioxo)-1,2,4-triazino-[2,1-a]-1,2,4-triazine-1,7-diones **(2a,b)** and 2-(5-phenylthiazol-2-yl)-3-phenyl-5-(p-nitrobenzylidene)-1,2,4-triazine-6-one **(5)** were prepared via cyclocondensation of 2-substituted-1,2,4-triazine **(1a,b)** with ethyl chloroacetate and phenacyl bromide in presence of fused sodium acetate. Alkylation of compounds **2a,b** with ethyl chloroacetate in dimethyl formamide yielded N-alkylated products **(3a,b).** Condensation of compound **2a** with aromatic aldehydes afforded the corresponding arylidene derivatives **(4a,b)**. N-acetyl derivative **(6)** was obtained via acetylation of compound **5** with acetic anhydride. The structures of the compounds were elucidated by using spectral and elemental analysis. All the prepared fused triazino- and thiazolyl-1,2,4-triazine were evaluated for their cytotoxicity against HepG-2 cell line. Some of newly synthesized 1,2,4-triazine derivatives emerged as a potential candidate for the development of future cytotoxic compounds.*

INTRODCUTION

Nitrogen and sulfur heterocycles have been under investigation for a long time because of their significant medicinal properties. 1,2,4-triazines and their derivatives have been widely studied in terms of their synthetic methodologies and reactivity since some of these derivatives were reported to have promising biological activities [1-4]. A survey of the literature revealed that 2,5-disubstituted-3,1-oxazolin-4-ones are the most common reagents used for the synthesis of 1,2,4-triazines and their derivatives [5-7]. 1,2,4-Triazine derivatives have been reported to possess a broad spectrum of biological activities, including antifungal [8,9], anti-HIV [10,12], anticancer [13,14], anti-inflammatory [15,16], analgesic [17] and antihypertensive activities [18]. Besides this, 1,2,4-triazines were used as herbicides, pesticides and dyes [19,20].

This prompted us to synthesize fused triazino derivatives of 1,2,4-triazines, thiazolyl derivatives of 1,2,4-triazines and evaluate them for antitumor activities.

MATERIALS AND METHODS

NMR spectra were recorded on a General Electric QE 300 instrument and chemical shifts were given with respect to TMS. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a Broad FTS7 (KBr). Mass spectra were obtained on a Jeol JMS D-300 spectrometer operating at 70 eV. Microanalysis were conducted using an Elemental analyser 1106. Melting points were determined on a Reichet Hot Stage and uncorrected.

Synthesis of 5-(*p*-nitrobenzylidene)-3-phenyl-2-substituted-1,2,4-triazine-6-one (1a,b)

A mixture of 5-(*p*-nitrobenzylidene)-2-phenyl-3,1-oxazole-4-one (0.01 mole), semicarbazide hydrochloride and/or thiosemicarbazide (0.01 mole) and fused sodium acetate (0.03 mole) in glacial acetic acid (30 ml) was heated under reflux for 4 hr, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and purified with ethanol to give **1a,b.**

5-(p-Nitrobenzylidene)-3-phenyl-2-(aminocarbonyl)-1,2,4-triazine-6-one (**1a**) as yellow crystals, yield 71%, m.p. 265 ^{o}C. IR (KBr): 3414, 3217 (NH_2), 3267 (NH), 1730, 1714 (C=O), 1637 (C=N), 1597, 1516 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 6.50 (s, 2H, NH_2), 7.36-8.59 (m, 10H, Ar-H and H-olefinic), 9.05 (br. s, 1H, NH) ppm. ^{13}C- NMR (DMSO-d_6): δ 169.45, 165.32 (C=O), 148.25, 147.90 (C=N), 140.87, 140.07, 139.49, 136.71, 134.76, 133.69, 133.29, 133.04, 129.89, 129.40, 129.30, 129.15, 128.87, 127.89, 127.44, 125.55, 125.28, 124.26 (C-aromatic, triazine and C-olefinic) ppm. MS: (m/z, %) = 352 (M^++1, 1.70), 351 (M^+, 11.70), 350 (M^+-1, 56.50), 335 (2.40), 334 (2.20), 320 (1.20), 319 (4.70), 309 (18.70), 308 (100), 294 (11.10), 293 (34.50), 291 (7.60), 263 (1.30), 262 (3.30), 261 (3.70), 247 (3.40), 246 (4.60), 245 (2.20), 236 (1.30), 233 (1.40), 232 (1.30), 218 (1.20), 217 (2.10), 205 (1.80), 204 (3.40), 203 (3.10), 176 (3.10), 175 (6.80), 162 (5.90), 161 (15.60), 151 (1.60), 150 (1.90), 149 (7.90), 147 (3.60), 132 (2.20), 131 (2.30), 129 (4.00), 120 (5.80), 119 (48.00), 117 (5.10), 106 (4.10), 105 (53.50), 104 (42.00), 103 (12.60), 101 (11.70), 91 (1.60), 90 (2.20), 89 (8.80), 78 (2.70), 77 (32.80), 76 (8.00), 75 (10.00), 52 (1.10), 51 (6.60). 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 (**1b**) as yellow crystals, yield 73%, m.p. 220 ^{o}C. IR (KBr): 3422, 3163 (NH_2), 3285 (NH), 1730 (C=O), 1637 (C=N), 1600, 1518 (C=C), 1492 (C=S), 1101 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 7.23–8.53 (m, 12H, Ar-H, H-olefinic and NH_2), 10.72 (s, 1H, NH), 11.71 (s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 184.97, 182.43 (C=S), 168.56 (C=O), 161.50 (C-O), 148.03, 141.19 (C=N), 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, 128.30, 128.16, 124.91, 124.49, 124.30 (C-aromatic, triazine and C-olefinic) ppm. MS (m/z, %) = 368 (M^++1, 6.50), 366 (M^+-1, 1.00), 340 (1.10), 339 (1.20), 324 (1.10), 319 (1.30), 308 (11.20), 295 (7.00), 294 (21.80), 293 (18.30), 280 (1.20), 279 (1.00), 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 (2.80), 162 (3.90), 161 (2.20), 159 (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), 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, 18.98.

Synthesis of 2-(*p*-nitrobenzylidene)-4-phenyl-5-substituted-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,7-dione (2a,b)

A mixture of 2-substituted-1,2,4-triazine-6-ones (**1a,b**, 0.01 mole), ethyl chloroacetate (0.01 mole) and fused sodium acetate (0.03 mole) in glacial acetic acid (30 ml) was heated under reflux for 4 hr. The reaction mixture was cooled and poured into ice-water. The resulting solid was filtered off, washed with water, dried and purified by suitable solvent to give **2a,b**.

2-(p-Nitrobenzylidene)-4-phenyl-triazino-[2,1-a]-1,2,4-triazine-1,5,7-trione (**2a**) as yellow crystals, yield 68%, m.p. 168^{o}C. IR (KBr): 3221 (NH), 1732-1710 (br. C=O), 1635 (C=N), 1588, 1514 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6): □□3.98 (s, 2H, NCH_2CO), 7.13-8.61 (m, 10H, Ar-H and H-olefinic), 12.21-12.23 (br. s, 1H, NH) ppm. . ^{13}C-NMR (DMSO-d_6): □ 172.34, 170.70, 163.97 (C=O), 147.75, 147.57, 141.16, 140.28, 133.68, 133.47, 133.17, 132.84, 130.92, 130.30, 129.57, 129.44, 129.26, 129.16, 128.81, 128.48, 128.28, 128.08, 124.26, 123.73, 122.03 (C-aromatic and C-triazine), 36.28 (NCH_2CO) ppm. MS (m/z, %) = 392 (M^++1, 790), 391 (M^+, 25.30), 380 (3.20), 379 (4.10), 351 (1.80), 350 (4.90), 336 (3.30), 335 (3.80), 334 (7.70), 321 (2.30), 320 (3.10), 314 (3.70), 310 (2.40), 309 (16.00), 308 (78.40), 294 (12.60), 293 (54.30), 292 (18.90), 291 (11.60), 277 (5.00), 276 (21.70), 264 (4.40), 263 (5.60), 262 (8.20), 261 (4.20), 248 (5.00), 247 (22.80), 246 (18.80), 236 (3.20), 234 (1.70), 233 (2.60), 232 (2.30), 218 (2.50), 217 (2.60), 216 (2.40), 204 (4.70), 203 (5.60), 202 (8.10), 191 (2.40), 190 (7.60), 189 (3.80), 188 (5.00), 178 (2.30), 177 (2.90), 175 (6.90), 179 (11.00), 163 (5.30), 162 (21.00), 161 (4.50), 152 (2.60), 151 (3.70), 150 (6.70), 148 (14.80), 147 (6.60), 145 (12.00), 136 (2.10), 135 (5.00), 132 (10.70), 131 (30.40), 129 (5.80), 128 (3.70), 120 (10.10), 119 (100), 116 (7.60), 115 (10.80), 111 (4.90), 106 (8.30), 105 (89.50), 104 (89.50), 103 (41.90), 102 (8.60), 101 (14.20), 91 (5.20), 90 (4.20), 89 (16.40), 78 (7.60), 77 (91.40), 76 (21.80), 75 (14.90), 72 (12.60), 69 (8.70), 64 (3.60), 63 (8.30), 57 (10.50), 51 (13.40). Anal. Calcd. for $C_{19}H_{13}N_5O_5$ (391): C, 58.31; H, 3.32; N, 17.90. Found: C, 58.23; H, 3.16; N, 17.77.

2-(p-Nitrobenzylidene)-4-phenyl-thioxo-triazino-[2,1-a]-1,2,4-triazine-1,7-dione (**2b**) as yellow crystals, yield 71%, m.p. 260^{o}C. IR (KBr): 3157 (NH), 1730-1705 (br. C=O), 1637 (C=N), 1595, 1516 (C=C), 1448 (C=S) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.94 (s, 2H, NCH_2CO), 7.35-8.59 (m, 10H, Ar-H and H-olefinic), 12.22 (br.s , 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 174.61 (C=S), 168.23, 166.03, 161.85 (C=O), 154.77, 148.62 (C-O), 140.80, 140.06, 139.65, 136.70, 134.75, 133.64, 133.39, 133.31, 129.88, 129.44, 129.36, 128.99, 128.87, 127.98, 127.44, 125.28, 125.18, 124.54, 124.36, 124.24 (C-aromatic, triazine and olefinic), 33.62 (NCH_2CO) ppm. Anal. Calcd. for $C_{19}H_{13}N_5O_4S$ (407): C, 56.02; H, 3.19; N, 17.20. Found: C, 55.98; H, 3.02; N, 17.01.

Synthesis of 2-(*p*-nitrobenzylidene)-4-phenyl-5-substituted-6-(ethoxycarbonyl)methyl-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,7-diones (3a,b)

A mixture of compounds **2a** and **2b** (0.01 mole) and ethyl chloroacetate (0.01 mole) in dimethyl formamide (30 ml) was heated under reflux for 2-3 hr, then cooled and poured into water. The product formed was filtered off, washed with water, dried and purified by recrystallization from ethanol to give **3a,b.**

2-(p-Nitrobenzylidene)-4-phenyl-6-(ethoxycarbonyl)methyl-1,2,4-triazino-[2,1-a]-1,2,4-triazine-1,5,7-trione (**3a**) as pale yellow crystals, yield 65%, m.p. 105^{o}C. IR (KBr): 1755, 1735 (C=O), 1635 (C=N), 1595, 1517 (C=C), 1107, 1028 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 7.31-8.61 (m, 10 H, Ar-H and H-olefinic), 4.19 (q, 2H, OCH_2), 3.98 (s, 2H, NCH_2CO), 2.50 (s, 2H, NCH_2CO), 1.16 (t, 3H, CH_3) ppm. ^{13}C-NMR (DMSO-d$_6$): δ 170.81, 168.50, 160.85 (C=O), 148.32, 140.29, 137.84, 133.96, 133.52, 133.14, 129.89, 129.34, 129.19, 128.90, 128.47, 128.27, 128.01, 127.45, 127.29, 126.70, 125.92, 124.32, 124.16 (C-aromatic, triazine and C-olefinic), 61.45 (OCH_2), 36.28, 24.96 (2 x NCH_2CO), 14.42 (CH_3) ppm. MS (m/z, %) = 478 (M^++1, 7.60), 477 (M^+, 27.80), 442 (6.60), 441 (22.90), 394 (3.90), 393 (3.90), 392 (11.80), 391 (16.40), 351 (7.20), 350 (29.70), 335 (3.20), 334 (4.60), 333 (3.10), 321 (4.50), 320 (3.80), 319 (4.20), 309 (10.00), 308 (50.30), 294 (14.60), 293 (65.40), 292 (23.70), 291 (15.20), 278 (2.20), 277 (6.10), 276 (27.10), 263 (3.00), 262 (3.10), 261 (3.80), 248 (5.70), 247 (27.40), 246 (24.50), 218 (2.70), 217 (3.00), 216 (3.10), 204 (5.40), 203 (6.30), 202 (6.70), 190 (9.00), 189 (4.40), 188 (6.10), 176 (5.30), 175 (7.90), 174 (8.30), 163 (4.60), 162 (24.40), 161 (10.30), 149 (14.30), 145 (10.20), 144 (7.90), 132 (10.20), 131 (30.00), 129 (5.10), 128 (3.80), 119 (41.10), 118 (5.70), 117 (7.50), 115 (9.30), 106 (7.50), 105 (97.20), 104 (100), 103 (45.40), 101 (14.80), 91 (3.80), 90 (4.80), 89 (17.30), 88 (6.60), 77 (56.80), 76 (21.10), 75 (14.90), 63 (7.40), 62 (3.20), 51 (9.70). Anal. Calcd for $C_{23}H_{19}N_5O_7$ (477): C, 57.86; H, 3.98; N, 14.67. Found: C, 57.63; H, 3.76; N, 14.48.

2-(p-Nitrobenzylidene)-4-phenyl-5-thioxo-6-(ethoxycarbonyl)methyl-triazino-[2,1-a]-1,2,4-triazine-1,7-dione (**3b**) as pale yellow crystals, yield 71%, m.p. 168^{o}C. IR (KBr): 1760-1707 (br. C=O), 1637 (C=N), 1597, 1516 (C=C), 1446 (C=S), 1107, 1026 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 1.13 (t, 3H, CH_3), 2.51 (s, 2H, NCH_2CO), 3.89 (s, 2H, NCH_2CO), 4.23 (q, 2H, OCH_2), 7.23-8.61 (m, 10H, Ar-H and H-olefinic) ppm. ^{13}C-NMR (DMSO-d$_6$): 167.92 (C=S), 166.73, 165.58, 164.69 (C=O), 148.65, 147.51 (C-O), 140.79, 139.63, 133.72, 133.62, 133.46, 133.29, 132.63, 132.09, 131.27, 131.00, 129.84, 129.64, 129.43, 129.21, 128.95, 128.65, 128.32, 127.97, 125.21, 124.53, 124.21, 124.06, 123.83 (C-aromatic, triazine and C-olefinic), 62.02 (OCH_2), 39.35 (NCH_2CO), 33.62 (NCH_2CO), 14.46 (CH_3) ppm. Anal. Calcd for $C_{23}H_{19}N_5O_6S$ (493): C, 55.98; H, 3.85; N, 14.20. Found: C, 55.71; H, 3.63; N, 14.02.

Synthesis of 8-arylidene-5-thioxo-4-phenyl-2-(*p*-nitrobenzylidene)-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,7-diones (4a,b)

A mixture of **2b** (0.01 mole), aromatic aldehydes (such as benzaldehyde and anisaldehyde, 0.01 mole) and piperidine (1 ml) was fused on a hot plate at 120-130^{o}C for 2 hr. The reaction mixture was dissolved in ethanol and acidified with dilute hydrochloric acid (2N). The crude product obtained was filtered off, washed with water, dried and purified by recrystallization with acetic acid to give **4a,b**.

8-Benzylidene-5-thioxo-4-phenyl-2-(p-nitrobenzylidene)-1,2,4-triazino-[2,1-a]-1,2,4-triazine-1,7-dione (**4a**) as yellow crystals, yield 74%, m.p. 270^{o}C. IR (KBr): 3210-2650 (br. OH), 1716 (C=O), 1643 (C=N), 1606, 1587 (C=C), 1446 (C=S), 1107, 1065 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 7.33-8.66 (m, 15H, Ar-H and H-olefinic), 12.78 (br. s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d$_6$): δ 167.76 (C=S), 165.12, 162.10 (C=O), 156.37 (C-O), 148.92, 148.68 (C=N), 140.80, 140.38, 136.31, 133.93, 133.70, 131.50, 130.55, 130.45, 130.39, 130.20, 129.75, 129.64, 129.38, 129.02, 128.98, 128.70, 128.45, 128.31, 126.18, 124.55, 124.29, 124.09, 123.15 (C-aromatic, triazine and C-olefinic) ppm. Anal. Calcd for $C_{26}H_{17}N_5O_4S$ (495): C, 63.03; H, 3.43; N, 14.14. Found: C, 62.98; H, 3.31; N, 14.02.

8-(p-Methoxybenzylidene)-5-thioxo-4-phenyl-2-(p-nitrobenzylidene)-1,2,4-triazino-[2,1-a]-1,2,4-triazine-1,7-dione (**4b**) as yellow crystals, yield 71%, m.p. 285^{o}C. IR (KBr): 3107 (NH), 3200-2597 (br. OH), 1712 (C=O), 1643 (C=N), 1593, 1570 (C=C), 1463 (C=S), 1022, 1008 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 3.85 (s, 3H, OCH_3), 7.01-8.71 (m, 15H, Ar-H and H-olefinic), 9.913 (br. s, 1H, NH), 12.51 (br. s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d$_6$): δ 168.30 (C=S), 165.10, 161.12 (C=O), 155.30 (C-O), 148.70, 148.52 (C=N), 140.48, 140.27, 136.21, 133.21, 132.41, 132.28, 131.43, 130.15, 130.12, 129.65, 129.53, 129.23, 129.00, 124.56, 124.32, 124.21, 124.01, 115.35, 114.98 (C-aromatic, triazine and C-olefinic), 55.93 (O-CH_3) ppm. Anal. Calcd for $C_{27}H_{19}N_5O_5S$ (525): C, 61.71; H, 3.62; N, 13.33. Found: C, 61.52; H, 3.37; N, 13.17.

Synthesis of 2-(5-phenylthiazol-2-yl)-3-phenyl-5-(*p*-nitrobenzylidene)-1,2,4-triazine-6-one (5)

A solution of compound **1b** (0.01 mole), phenacyl bromide (0.01 mole) and fused sodium acetate (0.03 mole) in acetic acid (50 ml) was heated under reflux for 4 hr, then cooled and poured into ice-water. The solid formed was filtered off, washed with water, dried and purified from ethanol to give **5** as pale yellow crystals, yield 73%, m.p.

193°C. IR (KBr): 3280 (NH), 1714 (C=O), 1627 (C=N), 1597-1558 (C=C). ^{1}H-NMR (DMSO-d_6): δ 7.25-8.61 (m, 16H, Ar-H, H-thiazole and H-olefinic), 12.60 (br. s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 168.19, 162.75 (C=O), 148.12, 147.57 (C=N), 141.29, 140.59, 139.08, 136.71, 134.93, 134.33, 133.85, 133.39, 129.88, 129.15, 128.87, 128.31, 128.13, 127.50, 126.05, 125.82, 125.28, 124.62, 124.36, 104.95 (C-aromatic, triazine and C-olefinic) ppm. MS (m/z, %) = 468 (M^++1, 8.00), 467 (M^+, 26.20), 336 (2.80), 325 (7.40), 324 (34.80), 310 (2.90), 309 (9.10), 308 (8.50), 294 (16.20), 293 (77.00), 292 (14.50), 279 (3.10), 278 (8.50), 277 (3.60), 263 (7.10), 262 (4.50), 248 (2.20), 247 (4.40), 246 (3.70), 204 (2.00), 203 (3.00), 202 (6.30), 190 (2.50), 189 (2.90), 177 (13.00), 176 (100), 175 (19.90), 174 (10.20), 163 (2.40), 162 (10.00), 161 (5.30), 149 (6.10), 148 (16.60), 147 (4.60), 135 (11.90), 139 (90.70), 129 (7.50), 121 (8.40), 119 (4.30), 117 (3.40), 116 (5.20), 105 (31.00), 104 (85.50), 103 (19.10), 102 (16.00), 91 (4.60), 90 (11.00), 89 (19.00), 77 (37.70), 76 (15.20), 75 (8.40), 63 (7.70), 62 (3.40), 51 (10.90). Anal. Calcd for $C_{25}H_{17}N_5O_3S$ (467): C, 64.24; H, 3.64; N, 14.94. Found: C, 64.03; H, 3.48; N, 14.77.

Synthesis of 1-acetyl-2-(5-phenylthiazol-2-yl)-3-phenyl-5-(*p*-nitrobenzylidene)-1,2,4-triazine-6-one (6)

A solution of **5** (0.01 mole) in acetic anhydride (25 ml) was heated under reflux for 2 hr, then cooled and poured into ice-water. The resulting solid was filtered off, washed with water, dried and purified by benzene to give **6** as pale yellow crystals, yield 61%, m.p. 165°C. IR (KBr): 1747, 1708 (C=O), 1643 (C=N), 1597, 1558 (C=C), 1444 (C=S), 1106, 1072 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, $COCH_3$), 7.23-8.66 (m, 16H, Ar-H, H-thiazole and olefinic) ppm. ^{13}C-NMR (DMSO-d_6): δ 168.20, 161.51 (C=O), 148.39, 147.56 (C=N), 140.24, 139.08, 137.95, 134.44, 134.03, 133.66, 131.15, 130.31, 129.81, 129.40, 129.02, 128.78, 128.58, 128.20, 128.13, 127.41, 127.22, 126.01, 125.76, 124.61, 124.15, 104.94 (C-aromatic, triazine, C-thiazole and olefinic), 21.56 ($COCH_3$) ppm. MS (m/z, %) = 510 (M^++1, 3.50), 509 (M^+, 9.00), 468 (24.00), 467 (77.20), 439 (2.20), 406 (1.10), 391 (1.30), 361 (1.00), 345 (1.30), 324 (3.40), 310 (2.00), 309 (4.20), 308 (4.00), 294 (14.10), 293 (57.20), 279 (3.00), 278 (11.20), 277 (4.90), 276 (3.30), 264 (1.40), 263 (3.50), 262 (3.00), 247 (4.10), 246 (4.00), 219 (2.10), 218 (7.00), 203 (3.60), 202 (3.60), 201 (2.10), 190 (3.10), 189 (2.50), 177 (7.00), 176 (37.30), 175 (23.70), 179 (16.10), 162 (7.60), 161 (2.90), 149 (5.70), 148 (23.70), 147 (9.30), 135 (16.90), 134 (57.90), 133 (9.00), 129 (14.70), 121 (7.10), 120 (3.40), 119 (3.40), 117 (3.90), 116 (6.90), 115 (7.90), 105 (57.50), 104 (100), 103 (37.60), 102 (21.60), 90 (10.40), 89 (21.80), 88 (9.50), 78 (6.60), 77 (62.50), 76 (27.40), 75 (16.80), 64 (5.40), 63 (13.80), 62 (8.40), 51 (26.00), 50 (17.20). Anal. Calcd for $C_{27}H_{19}N_5O_4S$ (467): C, 63.65; H, 3.73; N, 13.75. Found: C, 63.36; H, 3.56; N, 13.48.

RESULTS AND DISCUSSION

II-1) Chemistry

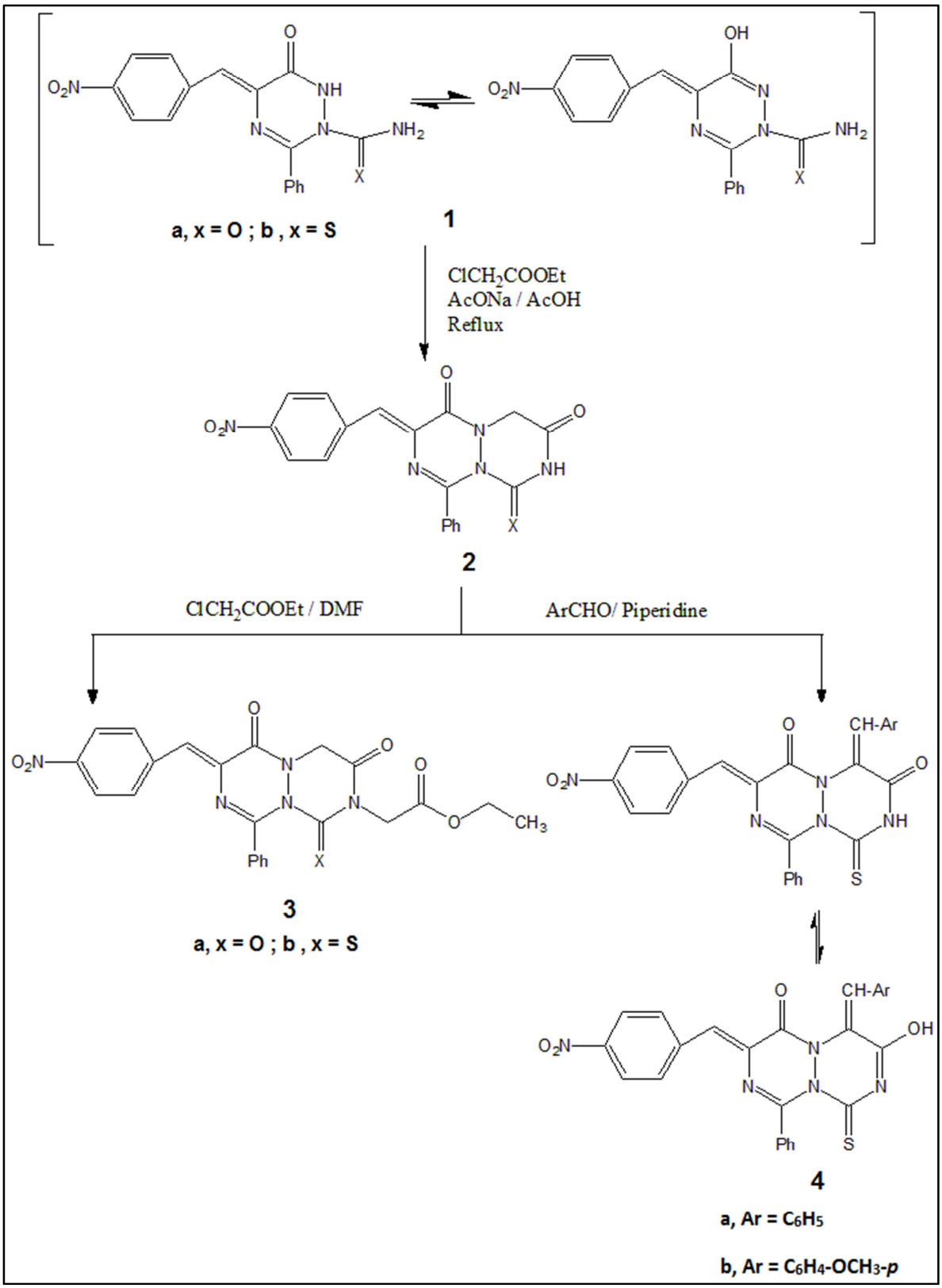
The 5-(*p*-Nitrobenzylidene)-3-phenyl-2-(aminocarbonyl) or (aminothiocarbonyl)-1,2,4-triazine-6-ones **(1a,b)** were prepared by cyclocondensation of the corresponding 5-(*p*-nitrobenzylidene)-2-phenyl-3,1-oxazol-4-one with semicarbazide hydrochloride and thiosemicarbazide in glacial acetic acid. The 2-substituted 1,2,4-triazine-6-ones **(1a,b)** were converted into the target 2-(*p*-nitrobenzylidene)-4-phenyl-5-oxo(thioxo)-1,2,4-triazino[2,1-*a*]-1,2,4-triazine-1,7-diones **(2a,b)** by the reaction with ethyl chloroacetate in the presence of fused sodium acetate and glacial acetic acid.

Alkylation of triazino-[2,1-*a*]-1,2,4-triazine-1,7-dione derivatives **(2a,b)** with ethyl chloroacetate in dimethyl formamide under reflux afforded the corresponding 2-(*p*nitrobenzylidene)-4-phenyl-5-oxo(thioxo)-6-(ethoxycarbonyl)methyl-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,7-diones **(3a,b)**.

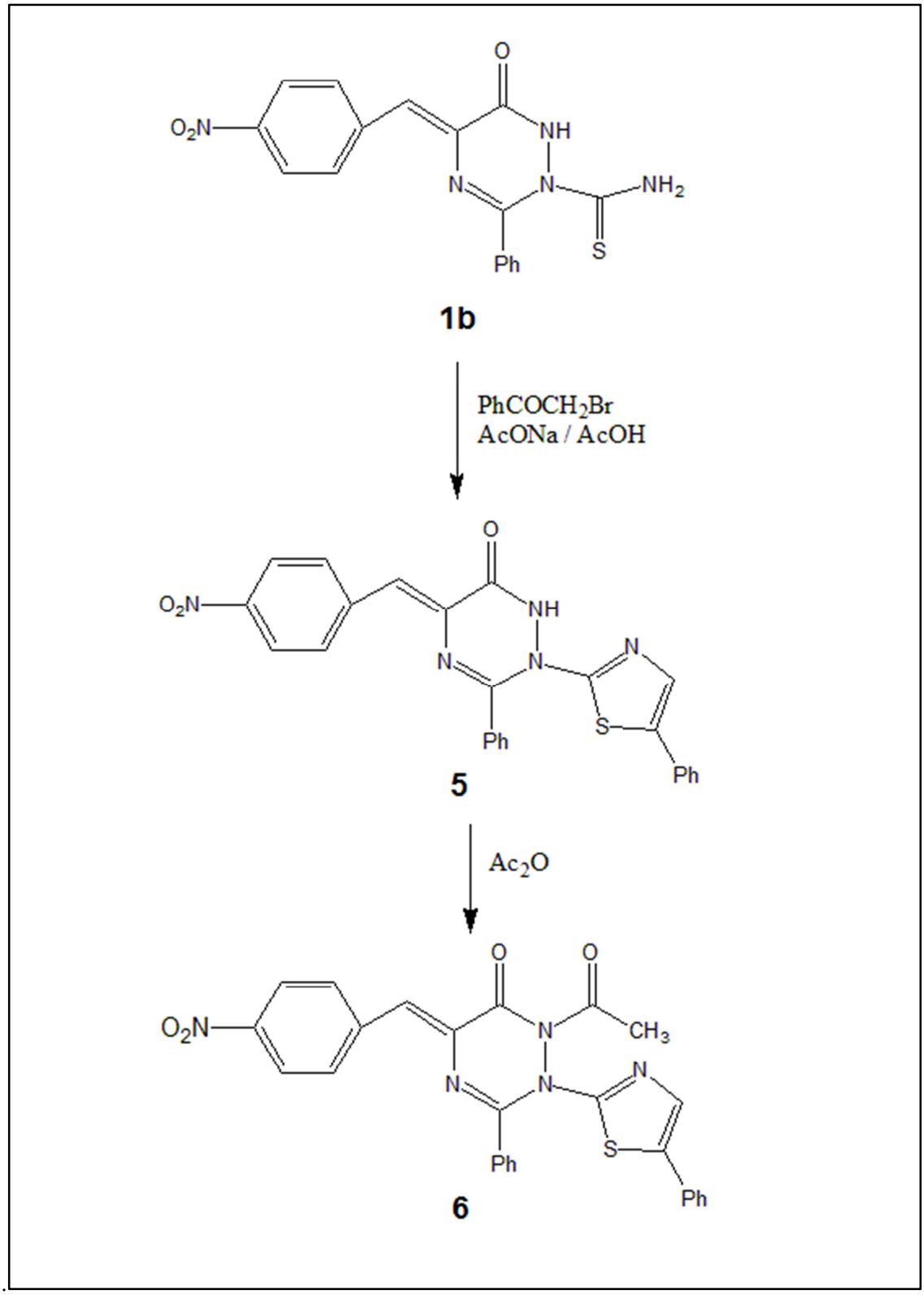
Condensation of triazino-[2,1-*a*]-1,2,4-triazine-1,7-dione derivative **(2a)** with aromatic aldehydes (such as benzaldehyde and anisaldehyde) in presence of piperidine under fusion yielded the corresponding 8-arylidene-5-thioxo-4-phenyl-2-(*p*-nitrobenzylidene)-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,7-diones **(4a,b)**. The structures of newly synthesized compounds **(Scheme 1)** were confirmed on the basis of their IR, NMR spectroscopic and EIMS spectrometry analysis as well as elemental analytical data.

The new compounds were synthesized by the general method in **scheme 2**, starting from 5-(*p*-nitrobenzylidene)-3-phenyl-2-(aminothiocarbonyl)-1,2,4-triazine-6-one **(1b)** that was converted into the corresponding 2-(5-phenylthiazol-2-yl)-3-phenyl-5-(*p*-nitrobenzylidene)-1,2,4-triazine-6-one **(5)** using phenacyl bromide as a reagent in presence of fused sodium acetate and glacial acetic acid. Treating the thiazolyl derivative **(5)** with acetic anhydride afforded the corresponding 1-acetyl-2-(5-phenylthiazol-2-yl)-3-phenyl-5-(*p*-nitrobenzylidene)-1,2,4-triazine-6-one **(6)**.

The structures of newly thiazole derivatives (**5** and **6**) were supported on the basis of their infrared, nuclear magnetic resonance spectroscopic and electron impact mass spectrometry analysis as well as elemental analytical data.


a, x = O ; b , x = S
1
ClCH2COOEt
AcONa / AcOH
Reflux
2
ClCH2COOEt / DMF
ArCHO/ Piperidine
3
a, x = O ; b , x = S
4
a, Ar = C6H5
b, Ar = C6H4-OCH3-p

Scheme 1



Scheme 2

II-2) Mass Spectrometry

The mass spectral decomposition mode [21,22] of 1,2,4-triazine heterocycles containing fused triazine and thiazolyl rings have been suggested and investigated.

Compounds 2a and 3a

The mass spectrum of 2-(*p*-nitrobenzylidene)-4-phenyl-1,2,4-triazino-[2,1-*a*]-1,2,4-triazine-1,5,7-trione **(2a) (Figure 1)** as example, showed intense molecular ion peak at *m/z* 391, consistent with molecular formula $C_{19}H_{13}N_5O_5$.

The molecular ion of compound **2a** fragmented to give peak at *m/z* 350 by losing ketene cation molecule (C_2HO). The loss of isocyanate group (NCO) from the fragment ion of *m/z* 350 produce the ion of *m/z* 308, corresponding to the molecular ion of 5-(*p*-nitrobenzylidene)-3-phenyl-1,2,4-triazine-6-one radical cation. The ion of *m/z* 308 underwent fragmentation and produce ion of *m/z* 293 by losing NH group. The ion of *m/z* 293 underwent fragmentation further and involved pathways as illustrated in **scheme 3**.

The mass spectra of compound **3a (Figure 2)** was fully consistent with assigned structure, intense molecular ion peak was observed. Thus compound **3a** showed an intense molecular ion peak at *m/z* 477, corresponding to the molecular formula $C_{23}H_{19}N_5O_7$.

The loss of lactone molecule ($C_4H_6O_2$) from the molecular ion peak of compound **3a** (*m/z* 477) gave a fragment ion of *m/z* 391, corresponding to the molecular ion of compound **2a**. The fragment ion of *m/z* 391 was broken via a pathway in the same fragmentation processes which was observed for compound **2a (Scheme 3)**. The EIMS of compound **2a** showed a base peak at *m/z* 119, while the compound **3a** showed a stable fragment at *m/z* 104.

Compounds 5 and 6

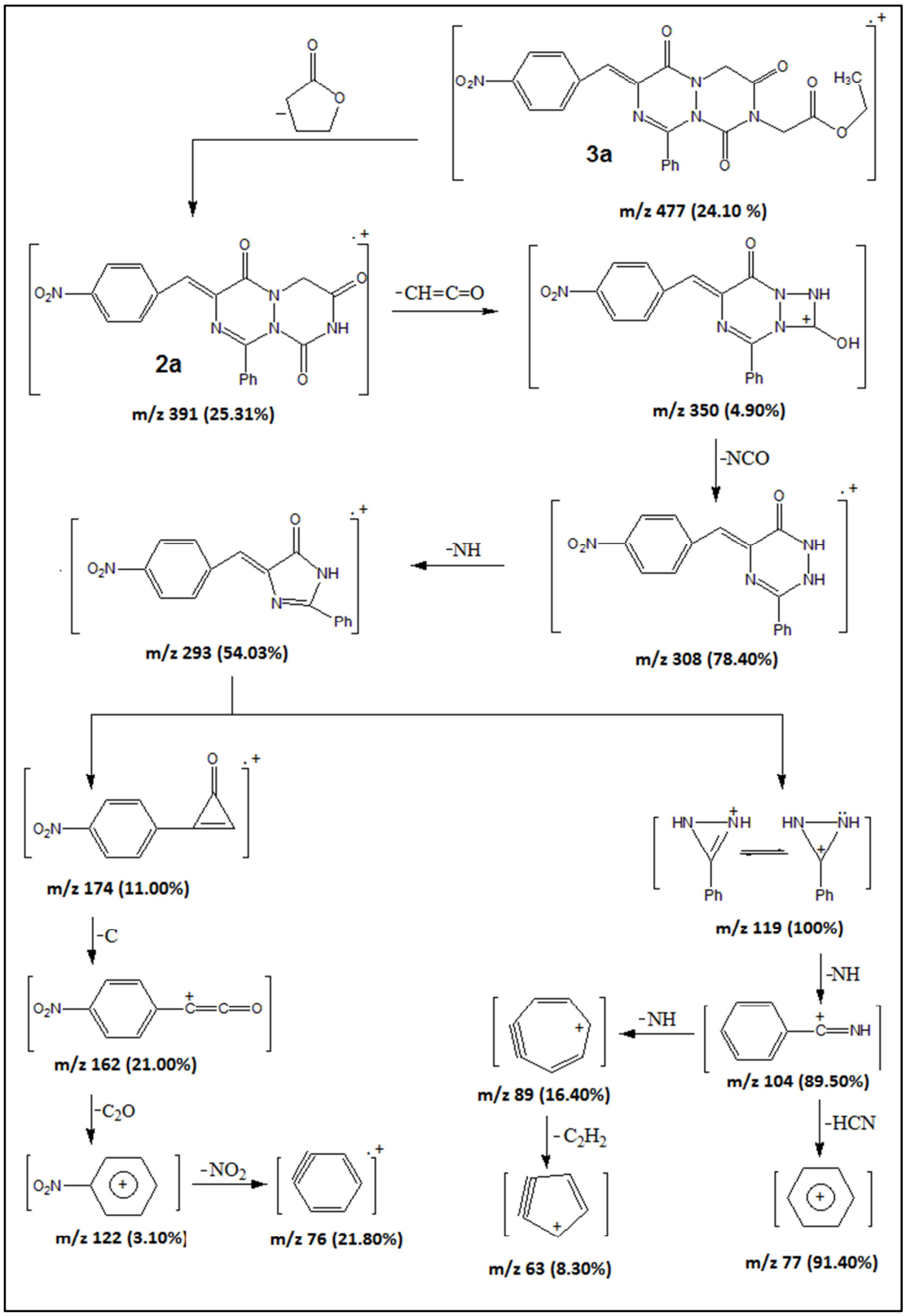
The mass spectra of compound **5** and **6** show relatively weak and strong molecular ions and peaks typical of a cleavage and rearrangement processes type fragmentation. Thus compound **5** and **6** showed an intense molecular ion peaks at *m/z* 467 and *m/z* 509, corresponding to the molecular formulas $C_{25}H_{17}N_5O_3S$ and $C_{27}H_{19}N_5O_4S$, respectively.

From the study of the mass spectrum of compound **5 (Figure 3)** it was found that the molecular ion for compound **5** at *m/z* 467 fragmented further and involved two various possible pathways as illustrated in **scheme 4**.

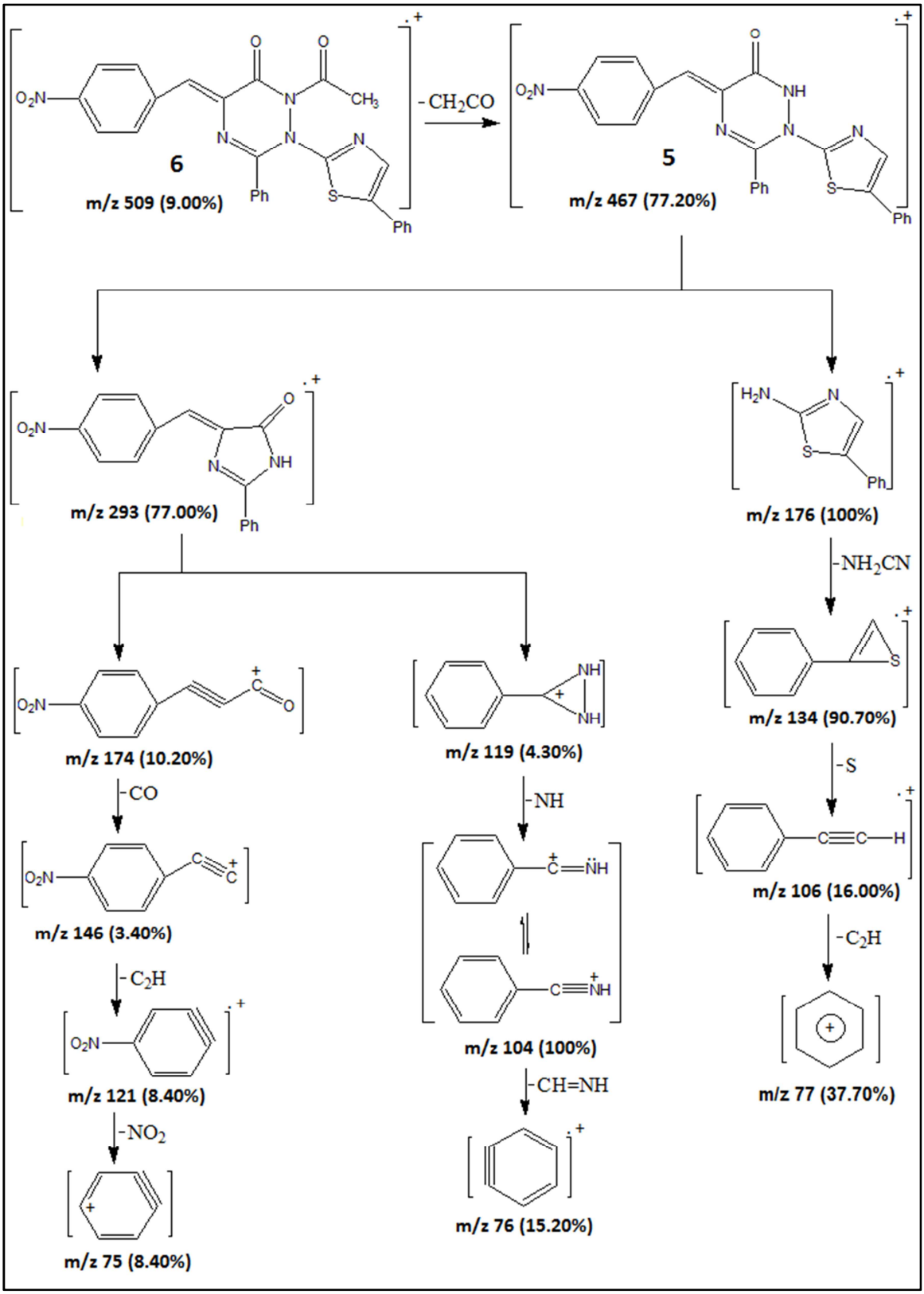
The molecular ion peak at *m/z* 467 for compound **5** fragmented to produce ion of *m/z* 176, corresponding to the 5-phenyl-2-aminothiazole radical cation. The loss of aminonitrile (NH_2CN) from the stable fragment ion of *m/z* 176 gave the fragment ion of *m/z* 134, which lost the sulphur atom and acetylene cation to give peaks at *m/z* 102 and *m/z* 77, respectively.

Subsequently, the molecular ion of compound **5** at *m/z* 467 was also found to undergo fragmentation to produce the ion of *m/z* 293, corresponding to 5-(*p*-nitrobenzylidene)-2-phenyl-imidazolidine-4-one radical cation. The fragment ion of *m/z* 293 fragmented further and involved two pathways as illustrated in **scheme 4**.

The molecular ion peak of compound **6** was observed at *m/z* 509 **(Figure 4)**. The loss of ketene molecule (CH_2CO) from the molecular ion peak at *m/z* 509 gave a strong fragment ion at *m/z* 467, corresponding to the molecular ion of compound **5**. The fragment ion of *m/z* 467 broke further via a pathway similar to compound **5 (scheme 4)**. The EIMS spectrum of compound **5** showed a stable fragment ion at *m/z* 176, while in case, the compound **6** gave the base peak at *m/z* 104.



Scheme 3: Main fragmentation pathway of compound 2a and 3a



Scheme 4 Main fragmentation pathway of compound 5 and 6

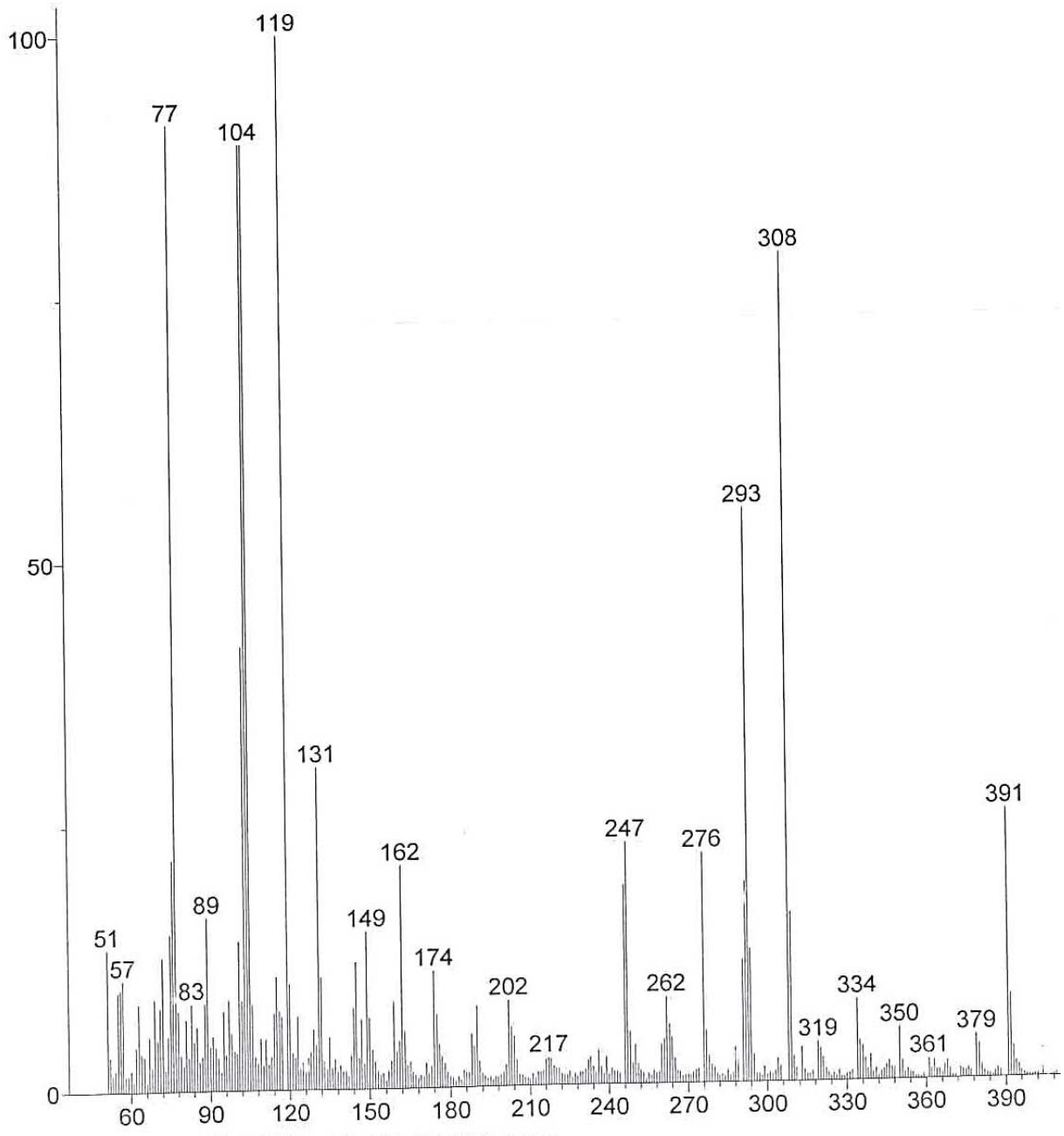


Figure 1: Mass spectrum of compound 2a

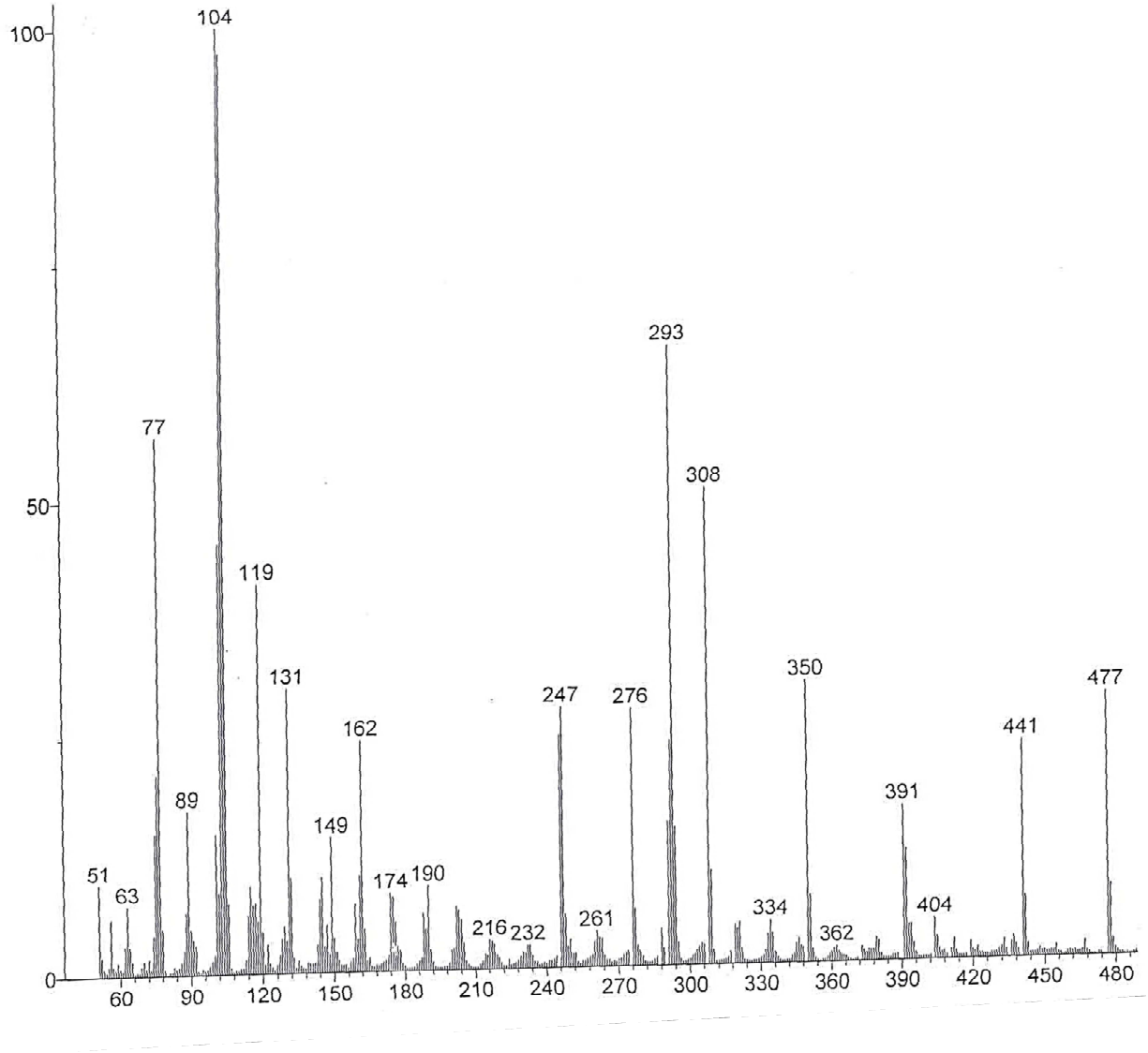


Figure 2: Mass spectrum of compound 3a

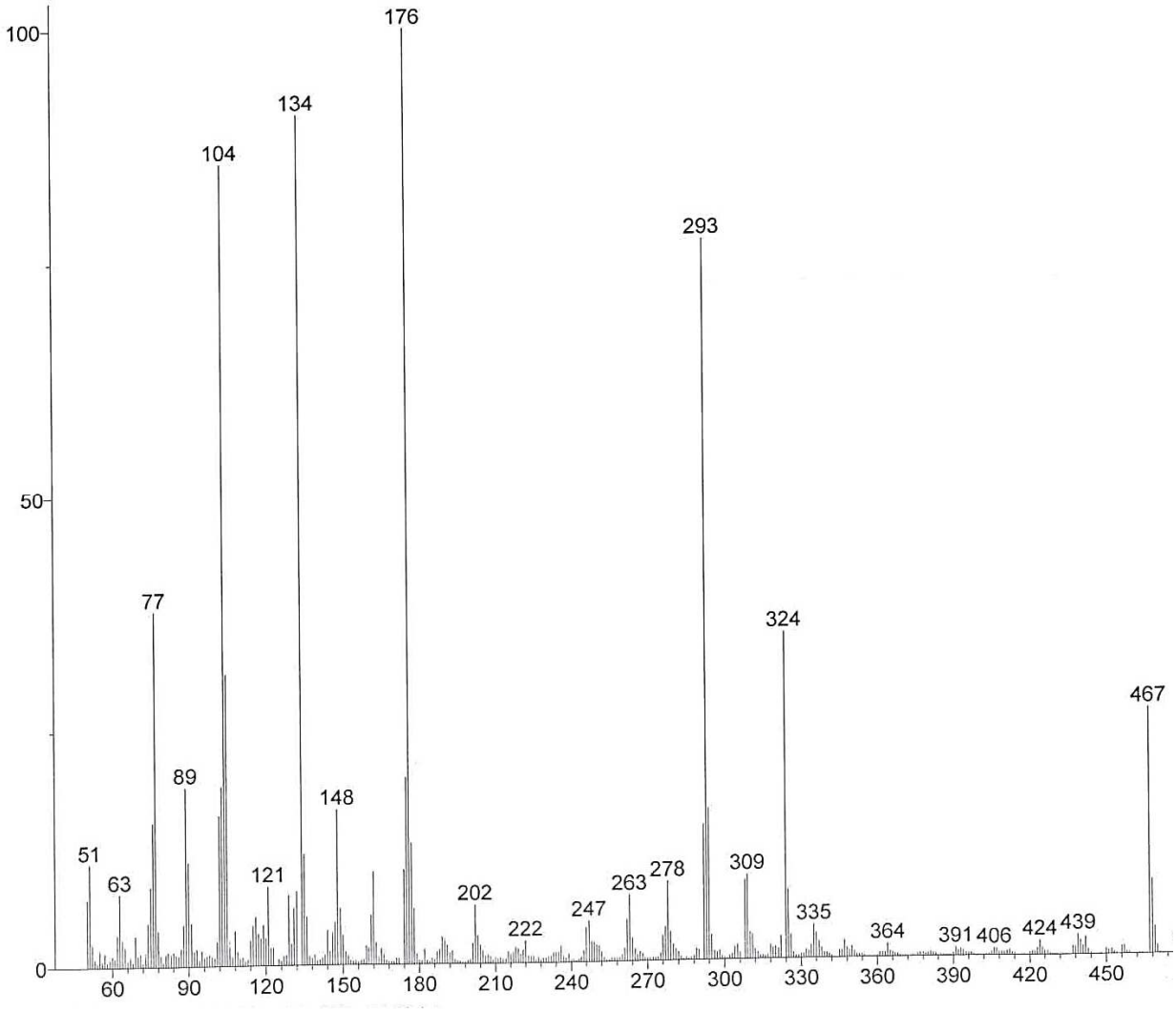


Figure 3: Mass spectrum of compound 5

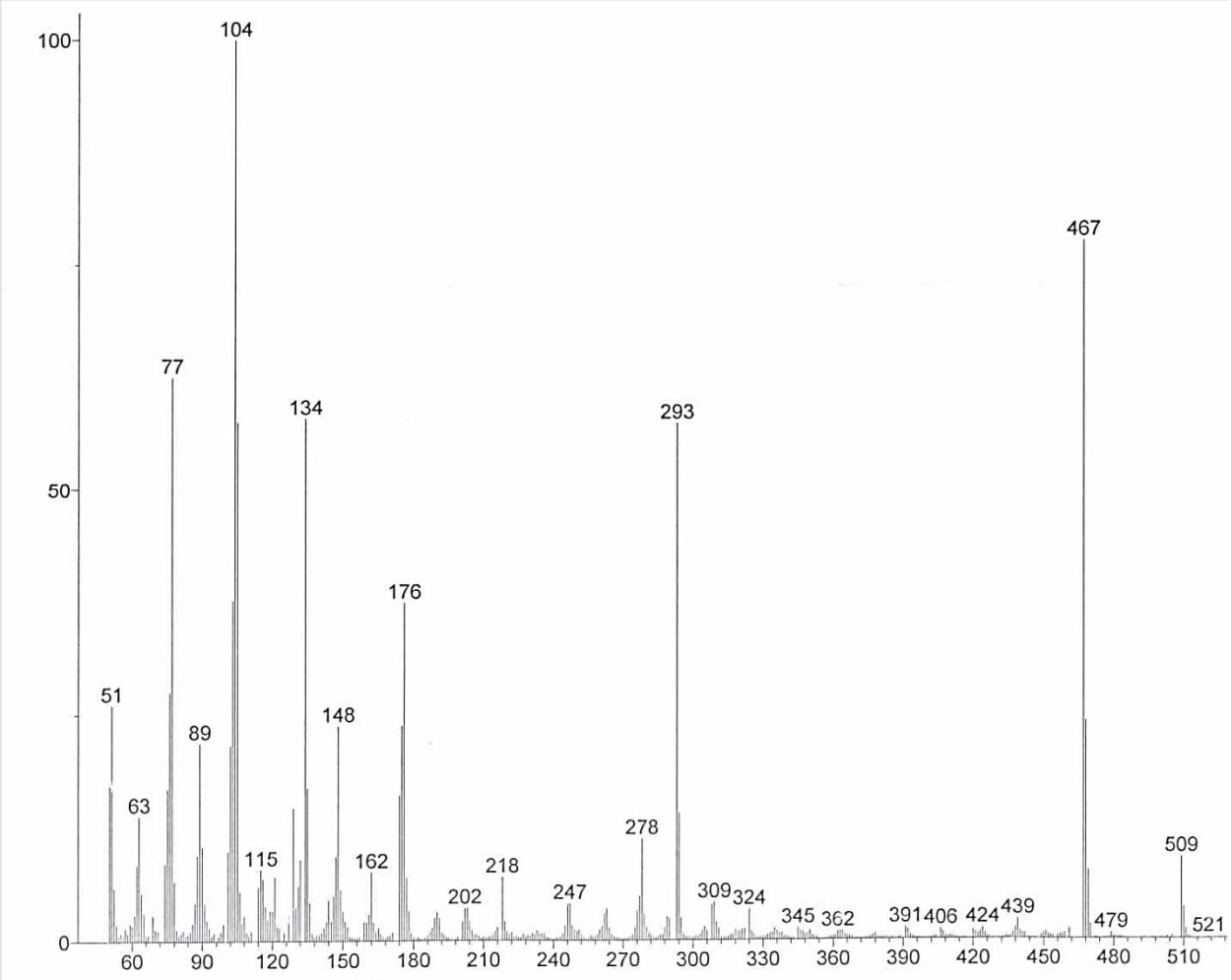


Figure 4: Mass spectrum of compound 6

II-3) Cytotoxicity Assay against Hepatocellular Carcinoma Cells

All the newly synthesized fused 1,2,4-triazino-1,2,4-triazine and thiazolyl-1,2,4-triazine derivatives were evaluated for their cytotoxicity against hepatocellular carcinoma cells (HepG-2 cell line) by Mosmann and Vijayan et al assay [23,24]. The 50% inhibitory concentration (IC_{50}) of HepG-2 cell lines was calculated from **table 1**, **Figure 5** and **6**.

Table 1: Evaluation of cytotoxicity of fused triazino-and thiazolyl-1,2,4-triazine derivatives against HepG-2 cell line.

Sample Concentration (µg)	Viability %								
	1a	**1b**	**2b**	**3b**	**4a**	**4b**	**5**	**6**	**Vinblastine standard**
50	18.74	7.45	10.32	21.65	32.78	10.94	20.38	28.21	7.82
25	29.16	15.39	17.63	35.16	45.07	18.42	27.06	36.87	15.18
12.5	41.53	22.76	24.18	60.47	69.42	24.31	38.94	49.76	29.60
6.25	62.47	31.49	32.94	75.28	81.59	38.53	59.61	72.94	48.75
3.125	79.41	40.75	45.21	82.44	92.68	53.91	78.24	81.52	60.35
1.56	88.15	59.27	61.89	90.25	98.73	74.85	86.93	90.63	76.24

The IC_{50} values are the concentration that induces 50% growth inhibition compared with untreated control cells.

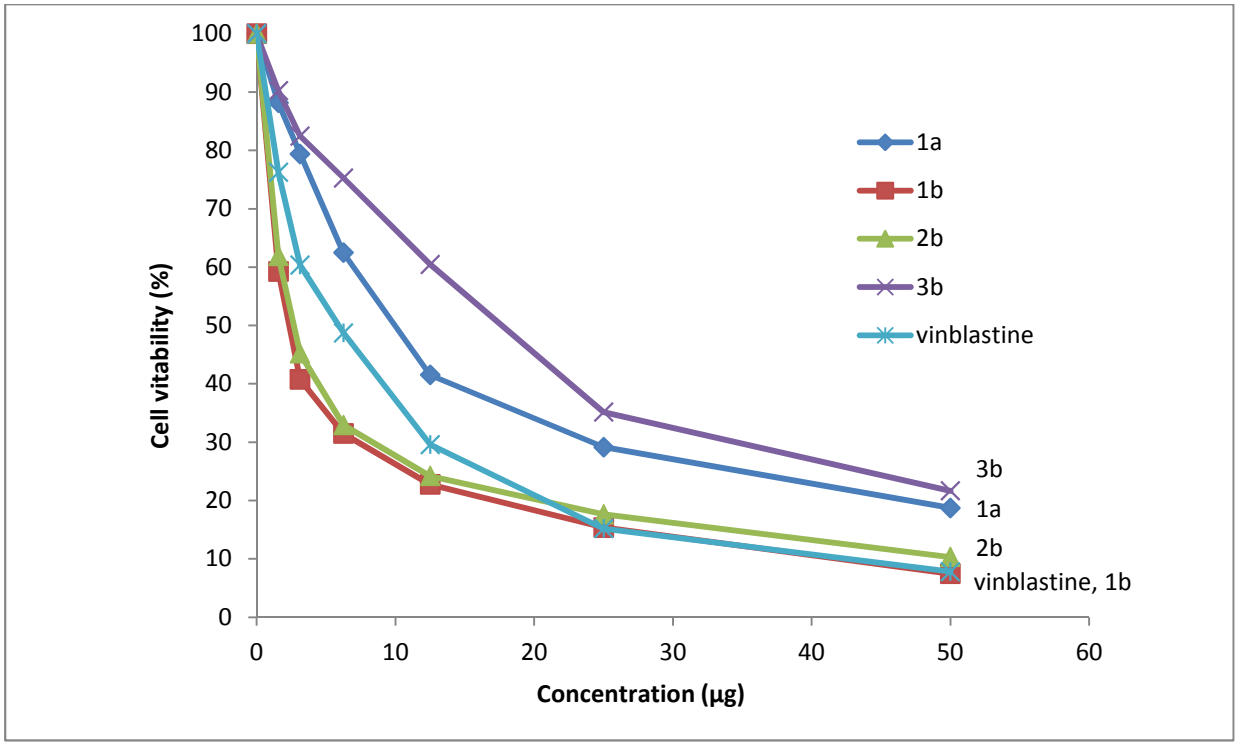


Figure 5: The inhibitory activities of compounds 1a, b, 2b and 3b

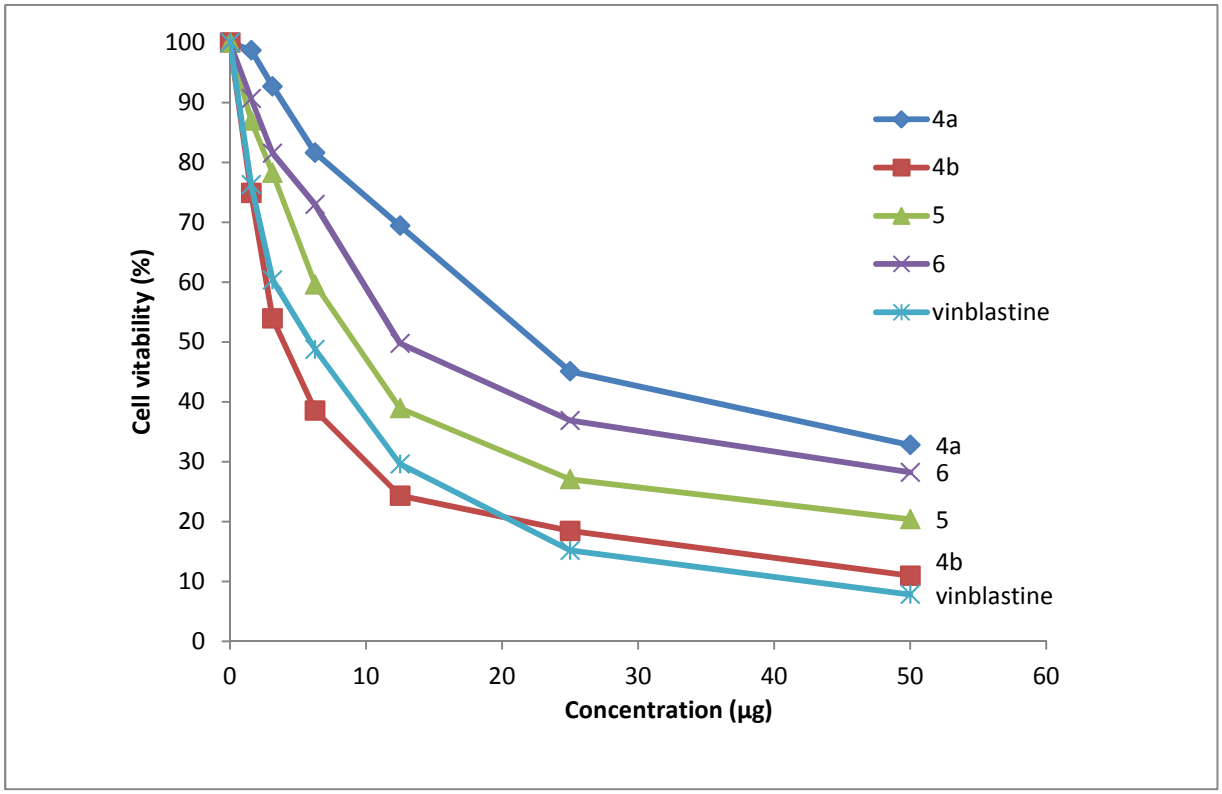


Figure 6: The inhibitory activities of compounds 4a, b, 5 and 6

The results of 50% inhibitory concentration (IC_{50}) data are summarized in **Table 2**

Table 2: IC_{50} (µg) values of synthesized compounds after 72 hour continuous exposure of tumour cell lines.

Compound number	1a	1b	2b	3b	4a	4b	5	6	Vinblastine standard
HepG-2 cell line	10.00	2.34	2.67	17.70	22.50	3.92	9.16	12.40	4.60

All the newly synthesized compounds have a cytotoxic effect against hepatocellular carcinoma cells. Compounds **1b**, **2b** and **4b** have a better cytotoxic effect than the corresponding compounds **1a**, **3b**, **4a**, **5** and **6**, comparing with the vinblastine drug assay.

CONCLUSION

The new fused triazino- and thiazolyl-1,2,4-triazine derivatives were synthesized using easy accessible methods, and their structures confirmed by IR, NMR spectroscopic and EIMS spectrometry, and elemental analysis. The synthesized 1,2,4-triazine derivatives have a cytotoxic effect against HepG-2 cell line. Furthermore the 1,2,4-triazine derivatives (**1b,2b** and **4b**) showed good activities than another triazine derivatives against HepG-2 cell line. Some 1,2,4-triazine derivatives were found to play an important role in medical chemistry as antitumor activity.

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