

Synthesis, Characterization and Biological Evaluation of Some Heterocyclic Compounds Based on 3-methoxy-2-hydroxybenzaldehyde

Amal Mahmoud Youssef Moustafa

Chemistry Department, Faculty of Science, Port-Said University, Port- Said, Egypt

Abstract: 8- Methoxy-3-acetylcoumarin, (2) was prepared via cyclocondensation of 3-methoxy-2-hydroxybenzaldehyde with ethyl acetoacetate in presence of piperidine. Upon condensation of 2 with thiosemicarbazide in methanol containing a catalytic amount of acetic acid, the corresponding derivative 3 was obtained in 86% yield. Compound 3, is the key intermediate for the synthesis of series of new compounds. Treatment of 3 with ω - bromomethyl aryl ketones in presence of fused sodium acetate yielded the corresponding thiazole derivatives 4(a, b). Reaction of 3 with oxazoles derivatives give the imidazolidinone derivatives 6(a,b). The electron impact ionization mass spectra of compounds 2 and 3 show a weak molecular ion peak and a base peak at m/z 203 and m/z 276 resulting from a cleavage fragmentation. The Compounds 4(a) and 5(b) give a characteristic fragmentation pattern with a very stable fragment at m/z 271 and m/z 318. In vitro antimicrobial activity of all synthesized compounds have been evaluated against five strains of bacterial culture, which includes three Gram +Ve bacterial culture such as *Bacillus Subtilis*, *Streptococcus Penumonia*, *Staphylococcus Aureas* and two Gram – Ve bacterial culture *Escherichia Coli* and *Pseudomonas Solanarium*, and two fungus such as *Aspergillus Niger* and *Penicillium Sp.* Almost compounds show very good activity. The others displayed moderate and mild activity. Compounds of 4-oxo-imidazolidin-2-thione derivatives have been tested for their antitumor activity against human breast carcinoma cell line (MCF-7). All tested compounds showed high activity against breast cancer with Median Inhibition Concentration (IC_{50}) range of 12.5-13 μ g/ml.

"1. Introduction"

Although, coumarins are a group of compounds which are naturally found in some plants, they can be synthetically produced as well. Because of their diverse derivatives, origin and properties most of them can be used for medicinal purposes. For example, they can be used against fungal diseases or in studying structure and biological properties of antifungal agents to discover new compounds with the similar activity [1]. Most fungi are completely

resistant to the action of antimicrobial drugs. Only a few substances have been discovered which exert an inhibitory effect on the fungi pathogenic for man, and most of these are relatively toxic [2].

Coumarin and its derivatives represent one of the most active classes of compound possessing a wide spectrum of biological activity [3-7]. Many of these compounds have proven to be active as antibacterial [8,9], antifungal [10], anti-inflammatory [11], and antitumor agents [12]. Thus it was thought worthwhile to synthesize some thiosemicarbazone, thiazoles and imidazolidinones containing coumarin moiety starting from 3-methoxy-2-hydroxybenzaldehyde as a key starting material.

"2. Materials and Methods"

Melting points were determined with a MEL-TEMP II apparatus and uncorrected. The IR spectra were recorded on Perkin Elmer 1420 Spectrometer and Biorad FTS7 (KBr). NMR spectra were recorded on general electric QE 300 instrument. Chemical shift values were reported in parts per million on the scale in dimethyl- d_6 sulfoxide with tetramethylsilane as the internal standard. Mass spectra were obtained on Joel JMS D-300 spectrometer operating at 70 eV. Microanalysis was conducted using an elemental analyzer, Heneaus CHN-OS Rapid.

8-Methoxy-3-acetylcoumarin (2)

A mixture of 3-methoxy-2-hydroxybenzaldehyde (0.01 mole), ethyl acetoacetate (0.01 mole) and piperidine (1ml) was fused on a hot plate for 3-5 min. The reaction mixture was added to boiling methanol (30 ml) and heated under reflux 2hrs, then cooled and poured into ice-HCl (2N). The Solid formed was filtered off, washed with water, dried and purified by recrystallization with methanol to give 2 as colorless crystals, yield 87%, m.p. 170°C. IR (KBr): 1725, 1695 (C=O), 1605, 1585 (C=C), 1215, 1170, 1085 (C-O) cm^{-1} . 1H -NMR (DMSO- d_6): δ 2.58 (s, 3H, COCH₃), 3.94(s, 3H, OCH₃), 7.30-7.49 (m, 3H, Ar-H), 8.61 (s, 1H, H-pyrane) ppm. MS: m/z (%) = 219 ($M^{+}+1$, 12.70), 218 (M^{+} , 68.00), 217 ($M^{+}-1$, 50.40), 204 (12.20), 203 (100), 202 (86.30), 176 (7.80), 175 (24.50), 174 (20.20), 148 (9.20), 147

(6.90), 146 (9.60), 132 (9.60), 131 (3.90), 130 (3.10), 120 (5.90), 119 (10.60), 118 (8.40), 117 (8.60), 105 (16.50), 104 (9.80), 103 (6.10), 91 (8.20), 90 (6.10), 89 (18.20), 88 (10.20), 77 (20.80), 76 (20.40), 65 (9.60), 64 (5.90), 63 (15.70), 51 (21.80), 50 (26.50). Anal. Found : C, 65.96; H, 4.43. C₁₂H₁₀O₄ requires: C, 66.06; H, 4.59.

8-Methoxy-3-acetylcoumarin thiosemicarbazone (3)

A mixture of 2 (0.01 mole), thiosemicarbazide (0.01 mole) and acetic acid (5ml) in methanol (30 ml) was heated under reflux for 2hrs, and then cooled. The solid formed was filtered, washed with methanol, dried and purified by recrystallization from ethanol to give 3 as yellow crystals, yield 86%, m.p. 205°C; IR (KBr): 3414, 3150 (NH₂), 3232 (NH), 1720 (C=O), 1618 (C=N), 1606, 1585 (C=C), 1325 (C=S), 1120, 1083 (C-O) cm⁻¹. ¹H-NMR (DMSO-d₆): δ 2.25 (s, 3H, CH₃), 3.92 (s, 3H, OCH₃), 7.29-7.33 (m, 3H, Ar-H), 7.97 (s, 2H, NH₂), 8.46 (s, 1H, H-pyrane), 10.46 (s, 1H, NH). MS: m/z (%) = 292 (M⁺+1, 11.30), 291 (M⁺, 71.10), 290 (M⁺-1, 49.00), 277 (15.50), 276 (97.90), 275 (20.30), 259 (3.40), 258 (8.10), 257 (4.70), 249 (21.60), 248 (18.10), 247 (9.50), 233 (5.10), 232 (22.70), 231 (46.00), 230 (38.70), 218 (10.90), 217 (21.50), 216 (49.60), 215 (40.00), 214 (23.20), 203 (12.10), 202 (17.30), 201 (27.60), 200 (22.90), 189 (14.80), 188 (11.20), 187 (8.80), 186 (8.30), 176 (8.70), 175 (11.80), 174 (14.50), 173 (16.30), 172 (13.60), 161 (19.00), 160 (20.40), 159 (14.30), 158 (11.80), 146 (9.20), 145 (14.10), 144 (10.80), 143 (7.60), 134 (4.70), 133 (10.00), 132 (13.60), 131 (18.70), 130 (19.60), 129 (10.50), 119 (17.90), 118 (11.30), 117 (17.30), 116 (24.30), 115 (34.50), 114 (14.10), 109 (25.60), 106 (5.10), 105 (12.90), 104 (17.30), 103 (35.40), 102 (24.80), 101 (11.50), 92 (10.00), 91 (23.40), 90 (48.10), 89 (52.50), 88 (20.60), 87 (11.90), 78 (16.60), 77 (66.20), 76 (56.50), 75 (39.40), 65 (33.70), 64 (18.00), 63 (47.00), 62 (33.10), 61 (14.00), 60 (100), 59 (56.00), 51 (60.20), 50 (57.00). Anal. Found: C, 53.52; H, 4.26; N, 14.23; S, 10.68. C₁₃H₁₃N₃O₃S requires: C, 53.61; H, 4.46; N, 14.43; S, 10.99.

5-Aryl-2-[[1-(8-methoxycoumarin-3-yl)ethylidene]hydrazino]-thiazoles 4(a,b)

A mixture of 3 (0.01 mole), ω- bromomethyl aryl ketones (such as 4-methylphenacylbromide and 4-methoxyphenacyl bromide) (0.01 mole) in acetic acid (30 ml) in presence of fused sodium acetate (0.03 mole) was heated under reflux for 4hrs, then cooled and poured into water.

The resulting solid was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound (4).

5-(4-Methylphenyl)-2-[[1-(8-Methoxycoumarin-3-yl)ethylidene]hydrazino]-thiazole 4(a) as yellow crystals, yield 78%, m.p. 165°C, IR (KBr): 3321 (NH), 1723 (C=O), 1625 (C=N), 1603, 1581 (C=C),

1120, 1083 (C-O) cm⁻¹. ¹H-NMR (DMSO-d₆): δ 2.25 (s, 3H, CH₃), 2.45 (s, 3H, CH₃), 3.91 (s, 3H, OCH₃), 7.21-7.85 (m, 8H, Ar-H and H-thiazole), 8.61 (s, 1H, H-pyrane), 10.60 (s, 1H, NH) ppm. MS: m/z (%) = 405 (M⁺, 7.50), 404 (M⁺-1, 6.50), 403 (M⁺-2, 8.40), 322 (3.70), 318 (7.50), 286 (3.70), 285 (5.60), 272 (17.80), 271 (100), 270 (91.60), 232 (9.30), 231 (11.20), 216 (14.00), 215 (11.20), 214 (7.50), 213 (2.80), 201 (8.40), 200 (10.30), 191 (9.30), 190 (20.60), 189 (20.60), 188 (16.80), 176 (13.10), 175 (16.80), 174 (11.20), 173 (8.40), 162 (5.60), 161 (10.30), 149 (13.10), 148 (38.30), 147 (24.30), 146 (24.30), 135 (11.20), 134 (17.80), 133 (11.20), 131 (12.10), 119 (13.10), 118 (12.10), 117 (15.90), 116 (27.10), 115 (29.90), 114 (23.40), 105 (4.00), 104 (15.00), 103 (15.90), 102 (16.80), 98 (15.90), 91 (35.50), 90 (29.90), 88 (11.20), 82 (30.80), 78 (14.00), 77 (20.60), 76 (15.00), 75 (13.10), 67 (26.20), 65 (17.80), 64 (19.60), 62 (19.60), 54 (21.10), 52 (15.90), 51 (15.00). Anal. Found: C, 65.03; H, 4.46; N, 10.21; S, 7.81. C₂₂H₁₉N₃O₃S requires: C, 65.18; H, 4.69; N, 10.37; S, 7.90.

5-(4-Methoxyphenyl)-2-[[1-(8-methoxycoumarin-3-yl)ethylidene]hydrazino]-thiazole 4(b) as yellow crystals, yield 73%, m.p. 170°C; IR (KBr): 3222 (NH), 1721 (C=O), 1623 (C=N), 1605, 1581 (C=C), 1215, 1071 (C-O) cm⁻¹. ¹H-NMR (DMSO-d₆): δ 2.50 (s, 3H, CH₃), 3.78-3.92 (s, 6H, 2xOCH₃), 7.31-7.91 (m, 8H, Ar-H and H-thiazole), 8.64 (s, 1H, H-pyrane), 10.46 (s, 1H, NH) ppm. MS: m/z (%) = 421 (M⁺, 21.20), 420 (M⁺-1, 22.20), 419 (M⁺-2, 33.30), 287 (44.40), 286 (66.70), 231 (44.40), 230 (13.20), 175 (16.20); 174 (11.20), 169 (11.10), 168 (33.30), 153 (5.60), 152 (27.80), 150 (38.90), 149 (27.80), 135 (44.40), 115 (27.80), 114 (55.60), 110 (38.90), 109 (27.80), 108 (22.20), 99 (22.20), 98 (61.20), 97 (27.80), 91 (27.80), 90 (50.00), 89 (83.30), 83 (38.90), 82 (55.60), 81 (61.10), 77 (61.10), 74 (50.00), 65 (55.60), 64 (83.30), 63 (61.10), 55 (100), 54 (50.00), 52 (77.80), 50 (44.4). Anal. Found: C, 62.52; H, 4.33; N, 9.78; S, 7.41. C₂₂H₁₉N₃O₄S requires: C, 62.71; H, 4.51; N, 9.98; S, 7.60.

5-Aryl-2-[[1-(8-methoxycoumarin-3-yl)ethylidene]acetylhydrazino]-thiazoles 5(a,b).

A solution of 4 (0.01 mole) in acetic anhydride (20 ml) was heated under reflux for 2hrs, then cooled and poured into ice-water. The solid obtained was filtered off, washed with water, dried and purified by recrystallization from benzene to give compound (5).

5-(4-methylphenyl)-2-[[1-(8-methoxycoumarin-3-yl)ethylidene]acetylhydrazino]-thiazole 5(a), as pale yellow crystals, yield 63%, m.p. 150°C. IR (KBr): 1721, 1695 (C=O), 1623 (C=N), 1605, 1583 (C=C), 1212, 1078 (C-O) cm⁻¹. ¹H-NMR (DMSO-d₆): δ 2.16 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 2.57 (s, 3H, COCH₃), 3.92 (s, 3H, OCH₃), 7.24-7.85 (m, 8H, Ar-H and H-olefinic), 8.61 (s, 1H, H-pyrane) ppm. MS: m/z (%) = 448 (M⁺+1), 3.20), 447 (M⁺, 6.20), 318 (2.30), 317 (1.30), 272 (11.20), 271 (63.20), 232

(12.20), 231 (25.50), 189 (25.50), 188 (13.50), 185 (10.60), 177 (31.90), 176 (17.00), 175 (14.90), 167 (10.60), 149 (48.90), 148 (57.40), 143 (25.50), 132 (10.60), 130 (10.60), 129 (14.90), 127 (14.90), 121 (17.00), 119 (74.50), 118 (34.00), 110 (17.00), 109 (74.50), 106 (23.40), 105 (12.80), 104 (19.10), 103 (17.00), 102 (19.10), 101 (10.60), 97 (23.40), 93 (917.00), 92 (17.00), 91 (48.90), 90 (25.50), 89 (14.90), 79 (44.70), 77 (19.10), 76 (29.80), 75 (21.30), 69 (38.30), 68 (40.40), 66 (36.20), 65 (31.90), 64 (25.50), 62 (12.80), 57 (53.20), 55 (63.80), 51 (34.00), 50 (23.40). Anal. Found: C, 64.22; H, 4.51; N, 9.19; S, 7.03. $C_{24}H_{21}N_3O_4S$ requires: C, 64.43; H, 4.70; N, 9.39; S, 7.16.

5-(4-Methoxyphenyl)-2-[[1-(8-methoxycoumarin-5-yl)ethylidene]acetylhydrazino]-thiazole 5(b) as pale yellow, yield 61%, m.p. 160°C. IR (KBr): 1723, 1647 (C=O), 1625 (C=N), 1607, 1589 (C=C), 1215, 1171, 1068, (C-O) cm^{-1} . 1H -NMR (DMSO- d_6): δ 2.15 (s, 3H, COCH₃), 2.58 (s, 3H, CH₃), 3.83-3.92 (s, 6H, 2xOCH₃), 7.16-7.74 (m, 8H, Ar-H and H-olefinic), 8.64 (s, 1H, H-pyrane). MS: m/z (%) = 464 ($M^+ + 1$, 1.30), 463 (3.50), 421 (1.80), 397 (5.70), 396 (3.10), 377 (6.20), 376 (8.80), 375 (15.00), 374 (15.90), 367 (10.10), 366 (10.10), 365 (10.60), 338 (7.00), 334 (5.70), 333 (14.50), 332 (11.90), 318 (100), 317 (85.50), 289 (7.90), 288 (12.80), 287 (48.90), 286 (12.80), 277 (10.60), 276 (35.70), 275 (33.90), 274 (5.20), 263 (11.90), 262 (6.20), 259 (9.50), 258 (11.90), 248 (9.70), 247 (10.10), 232 (16.30), 231 (28.20), 230 (29.50), 218 (11.50), 216 (20.70), 215 (19.80), 214 (18.50), 203 (22.40), 202 (13.20), 201 (20.30), 200 (16.30), 206 (13.20), 205 (8.40), 190 (12.30), 189 (15.40), 188 (13.20), 176 (15.90), 174 (11.00), 173 (8.80), 164 (15.40), 163 (11.50), 161 (11.00), 160 (12.30), 159 (15.40), 158 (14.80), 157 (15.00), 148 (13.20), 147 (11.00), 146 (8.80), 144 (15.40), 134 (25.10), 132 (17.60), 131 (18.10), 130 (14.10), 119 (11.90), 118 (14.50), 117 (13.20), 116 (16.30), 114 (16.70), 105 (9.30), 104 (10.60), 103 (17.60), 102 (16.70), 98 (16.70), 92 (11.90), 91 (11.90), 90 (14.10), 89 (18.90), 88 (11.50), 78 (15.00), 77 (35.20), 76 (22.50), 75 (17.60), 69 (12.30), 65 (15.40), 64 (14.10), 63 (28.60), 60 (38.30), 59 (23.80), 51 (26.50), 50 (24.70). Anal Found: C, 62.01; H, 4.31; N, 8.97; S, 6.69. $C_{24}H_{21}N_3O_5S$ requires: C, 62.20; H, 4.53; N, 9.07; S, 6.91.

3-[[1-(8-Methoxycoumarin-3-yl)ethylidenehydrazino]thiocarbonyl]-2-aryl-5-benzylidene-4-oxo-imidazolidines 6(a,b).

A mixture of **3** (0.01 mole) and oxazole derivatives (such as 5-benzylidene-4-oxo-2-phenyl-3,1-oxazolinine and 5-benzylidene-4-oxo-2-(*p*-chlorophenyl)-3,1-oxazolinone) (0.01 mole) in dimethyl formamide (30 ml) was heated under reflux for 4hrs, then cooled and poured into water. The solid formed was collected by filtration, washed with water, dried

and purified by recrystallization with ethanol to give compound (**6**).

2-Phenyl-3-[[1-(8-methoxycoumarin-3-yl)ethylidenehydrazino]thiocarbonyl]-4-oxo-5-benzylidene-imidazolidinone 6(a) as pale yellow crystals, yield 67%, m.p. 138°C. IR (KBr): 3260 (NH), 1723, 1697 (C=O), 1623 (C=N), 1605, 1601, 1593 (C=C), 1319 (C=S), 1225, 1081 (C-O) cm^{-1} . 1H -NMR (DMSO- d_6): δ 2.50 (s, 3H, CH₃), 3.89 (s, 3H, OCH₃), 7.22-7.85 (m, 14H, Ar-Hand olefinic-H), 8.50 (s, 1H, H-pyrane), 10.62 (s, 1H, NH) ppm. MS: m/z (%) = 522 (M^+ , 0.7), 521 (0.7), 408 (0.6), 407 (0.70), 368 (1.20), 353 (1.50), 352 (1.70), 317 (1.90), 316 (1.70), 303 (1.20), 302 (1.10), 301 (2.10), 300 (1.60), 287 (0.60), 286 (1.50), 285 (3.40), 284 (2.80), 283 (8.30), 282 (4.80), 273 (1.10), 272 (0.90), 257 (0.90), 249 (1.10), 248 (1.00), 232 (3.80), 231 (2.40), 230 (2.20), 217 (1.10), 216 (1.20), 215 (2.20), 203 (1.10), 202 (1.80), 201 (1.20), 188 (0.70), 186 (0.90), 175 (1.00), 163 (1.00), 162 (1.10), 161 (1.20), 159 (1.30), 157 (1.20), 146 (0.90), 145 (1.10), 136 (1.30), 135 (1.50), 131 (1.20), 127 (1.00), 125 (1.70), 123 (2.80), 122 (2.10), 119 (1.10), 118 (1.10), 106 (10.00), 105 (100), 104 (76.70), 103 (3.30), 94 (1.10), 91 (1.10), 90 (1.50), 89 (3.70), 88 (2.90), 78 (6.50), 77 (54.30), 76 (41.60), 69 (2.00), 65 (1.70), 64 (3.10), 63 (3.80), 62 (2.60), 51 (21.20), 50 (11.60). Anal Found: C, 66.39; H, 4.03; N, 10.58; S, 6.02. $C_{29}H_{22}N_4O_4S$ requires: C, 66.66; H, 4.21; N, 10.73; S, 6.13.

2-(*p*-Chlorophenyl)-3-[[1-(8-methoxycoumarin-3-yl)ethylidene hydrazino]thiocarbonyl]-4-oxo-5-benzylidene-imidazolidone 6(b) as yellow crystals, yield 63%, m.p. 153°C. IR (KBr): 1722, 1693 (C=O), 1624 (C=N), 1610, 1603, 1583 (C=C), 1317 (C=S), 1287, 1081 (C-O) cm^{-1} . 1H -NMR (DMSO- d_6): δ 2.45 (s, 3H, CH₃), 3.88 (s, 3H, OCH₃), 7.12-7.72 (m, 13H, Ar-H and H-olefinic), 8.54 (s, 1H, H-pyrane), 10.56 (s, 1H, NH) ppm. MS: m/z (%) = 557 ($M^+ + 1$, 0.30), 556 (M^+ , 0.070), 539 (30.40), 538 (21.70), 537 (26.10), 416 (26.10), 415 (34.80), 355 (30.40), 350 (26.10), 349 (30.40), 345 (21.70), 335 (43.50), 334 (34.80), 333 (30.40), 318 (43.50), 317 (52.20), 305 (26.10), 304 (47.80), 302 (34.80), 300 (60.90), 299 (30.40), 284 (21.70), 283 (26.10), 274 (17.40), 265 (30.40), 264 (21.70), 253 (26.10), 234 (39.10), 233 (34.80), 231 (13.00), 217 (73.90), 216 (13.00), 215 (30.40), 214 (30.40), 203 (26.10), 202 (60.90), 201 (100), 200 (73.90), 189 (52.20), 188 (26.10), 186 (43.50), 185 (34.80), 176 (52.20), 175 (56.50), 174 (34.80), 161 (69.60), 160 (47.80), 159 (39.10), 149 (43.50), 148 (43.50), 147 (82.60), 146 (52.20), 136 (34.80), 135 (52.20), 134 (43.50), 133 (21.20), 132 (73.90), 131 (30.40), 130 (52.20), 129 (60.90), 128 (60.90), 121 (60.90), 120 (43.50), 119 (43.50), 118 (78.30), 117 (26.10), 114 (39.10), 104 (43.50), 103 (78.30), 101 (73.90), 92 (21.70), 91 (39.10), 90 (26.10), 89 (39.10), 80 (87.70), 78 (17.40), 73 (78.30), 67 (73.90), 65 (34.80), 64 (52.20), 63

(17.40), 57 (39.10), 55 (73.90), 54 (56.80), 53 (17.40). Anal. Found: C, 62.33; H, 3.55; N, 9.98; S, 5.61. $C_{29}H_{21}N_4ClO_4S$ requires: C, 62.59; H, 3.77, N, 10.07; S, 5.76.

Biological Evaluation:

1. Antimicrobial activity:

The antimicrobial activity was assayed by cup plate agar diffusion method [13,14], by measuring inhibition zones in mm. *In vitro* antimicrobial activity of all synthesized compounds have been evaluated against five strains of bacterial culture, which includes three Gram +Ve bacterial culture such as *Bacillus subtilis* (*B. subtilis*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Staphylococcus aureus* (*S. aureus*) and two Gram -Ve bacterial culture *Escherichia coli* (*E. coli*) and *Pseudomonas solanarium* (*P. solanarium*), and two fungus such as *Aspergillus niger* (*A. niger*) and *Penicillium sp.*

2. Antibacterial activity

The purified products were screened for their antibacterial activity by using cup-plate agar diffusion method. The nutrient agar broth prepared by the usual method, was inoculated aseptically with 0.5 ml of 24 hrs. old subculture of *B. subtilis*, *S. pneumoniae*, *S. aureus*, *E. coli* and *P. solanarium* in separate conical flasks at 40-50°C and mixed well by gentle of the flask were poured and evenly spread in petridish (90 mm in diameter) and allowed to set for two hrs. The cups (10 mm in diameter) were formed by the help of borer in agar medium and filled with 0.1 ml (100 µg/ml) solution of sample in DMF. The plates were incubated at 37°C for 24 hrs and the control was also maintained with 0.1 ml of DMF in similar manner and the zones of inhibition of bacterial growth were measure in millimeter.

3. Antifungal activity:

A. niger and *Penicillium sp.* were employed for testing antifungal activity by cup-plate agar diffusion method [15-17]. The culture was maintained on sub rouse dextrose agar slants. Sterilized sub rouse dextrose agar medium was inoculated with 72 hrs old 0.5 ml suspension of fungal spores in a separate flask. About 25 ml of the inoculated medium was evenly spread in sterilized petridish and allowed to set for 2 hrs. The cups (10 mm in diameter) were punched in petridish and loaded with 0.1 ml (100 µg/ml) of solution of sample in DMF. The plates were inoculated at 30°C for 48 hrs. After the completion of incubation period, the zones of inhibition of growth in the form of diameter in mm were measured. Along the test solution in each petridish one cup was filled up with solvent which acts as control.

4. Measurement of Potential Cytotoxicity by SRB assay:

Potential cytotoxicity of the compounds **5(b)** and **7(b)** was measured in National Cancer Institute, Cancer Biology Department, Pharmacology Unit, Cairo University. The Potential cytotoxicity of the previous compounds against MCF-7 cells was determined using Sulfo-rhodamine-B assay [18]. In brief, tumor Cells were seeded into 96-multiwell microtiter plates at a concentration of 5×10^4 cells/well in fresh medium and left to attach to the plate for 24hrs before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Cells were then incubated for 48 hrs at 37°C and in atmosphere of 5% CO₂ in the absence (control) and in the presence of each compound at the noted concentrations (0, 1, 2.5, 5 and 10 µg/ml). Following 48 hrs exposure to the compounds, cells were fixed with 50% cold TCA for 1 h, stained for 30 min with 0.4% Sulfo-Rhodamine-B and then washed with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. The plates were then air-dried and the optical density of each well was measured spectrophotometrically at 564 nm using the EISA microplate reader (Meter tech. © 960, USA). Surviving fraction for each cell type was performed from which IC₅₀ was calculated for each compound under investigation. Worth mentioning is that the cytotoxic activity of Doxorubicin, a standard and well known anticancer drug, against the cell lines was performed at the same concentrations of tested compounds. The relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.

"3. Results and Discussion"

Cyclocondensation of 3-methoxy-2-hydroxybenzaldehyde **1** with ethyl acetoacetate in presence of piperidine afforded the corresponding 8-methoxy-3-acetylcoumarin thiosemicarbazone (**2**).

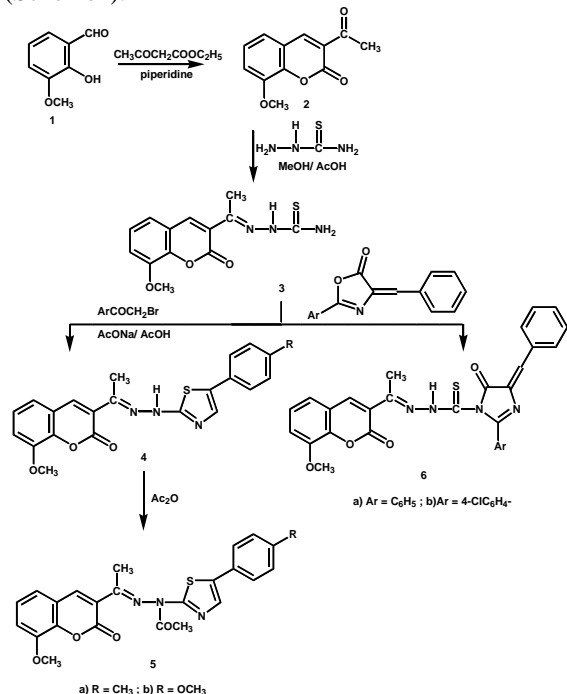
Condensation of 8-methoxy-3-acetylcoumarin (**2**) with thiosemicarbazide in methanol in presence of acid medium yielded the corresponding 8-methoxy-3-acetylcoumarin thiosemicarbazone (**3**).

The reaction of thiosemicarbazone (**3**) with ω-bromomethyl aryl ketones (such as 4-methylphenacyl bromide and 4-methoxyphenacyl bromide) in presence of fused sodium acetate in acetic acid under reflux gave the corresponding 5-aryl-2-[1-(8-methoxycoumarin-3-ylethylidene)hydrazino]-thiazoles **4(a,b)**.

Acetylation of thiazole derivatives (**4**) with acetic anhydride under reflux led to the formation 5-aryl-2-[1-(8-methoxycoumarin-3-ylethylidene)-acetylhydrazino]-thiazoles **5(a,b)**; (Scheme 1).

Treatment of 8-methoxy-3-acetylcoumarinthiosemicarbazone **3** with oxazole derivatives (such as 5-benzylidene-4-oxo-2-phenyl-3,1-oxazolinone and 5-benzylidene-4-oxo-2-(p-

chloro) phenyl-3,1-oxazolinone) in dimethyl formamide under reflux yielded the corresponding 3-[1-(8-methoxycoumarin-3-ylethylidenehydrazino)thiocarbonyl]-2-aryl-5-benzylidene-4-oxo-imidazolidinones **6(a,b)**; (Scheme 1).



Scheme 1

Mass Spectrometry

The mass spectral decomposition modes of the prepared coumarin derivatives have been investigated [12,19]. The mass spectrum of compound **2** (Figure 1) showed an intense molecular ion peak at m/z 218, corresponding to the molecular formula $C_{12}H_{10}O_4$. The molecular ion of compound **2** (Scheme 2) underwent fragmentation to produce a stable fragment of m/z 203 by losing methyl group. The loss of two carbonyl group ($C=O$) from the stable ion with m/z 203 resulted in ion of m/z 175 and m/z 147. The ion of m/z 147 underwent loss of carbon monoxide ($C=O$) and acetylene molecule to give peaks at m/z 119 and m/z 93, respectively.

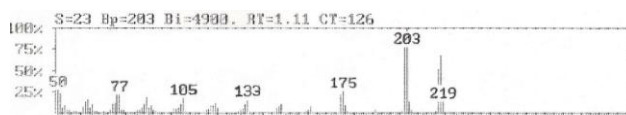
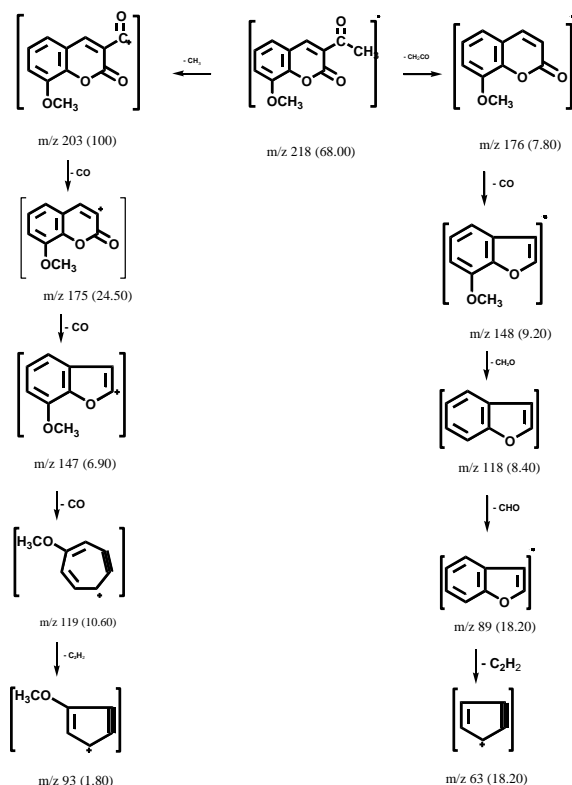


Figure 1. Mass Spectral Fragmentation pattern of compound 2



Scheme 2. Main fragmentation pathway of compound 2

Also the molecular ion for compound **2** underwent loss of ketone molecule (CH_2CO) to give peak at m/z 176. The loss of carbon-monoxide (CO) and formaldehyde (CH_2O) from the ion with m/z 176 resulted in ions at m/z 148 and m/z 118. The ion at m/z 118 underwent loss of formyl group to give peak at m/z 89. The ion of m/z 89 broke to give an ion at m/z 63 which lost acetylene molecule.

The mass spectrum of compounds (Figure 2) shows the molecular ion peak at m/z 291, corresponding to the molecular formula $C_{13}H_{13}N_3O_3S$. The molecular ion of compound **3** underwent fragmentation (Scheme 3) to produce the stable peak at m/z 276 by losing NH group. The loss of thioformaldehyde (CH_2S) from the ion with m/z 276 gave ion at m/z 230. The loss of nitrogen (N_2) and carbon dioxide (CO_2) from the ion with m/z 230 resulted in ions at m/z 202 and m/z 158. The ion of m/z 158 underwent loss of methoxy group (OCH_3), acetylene molecule (C_2H_2) and C_2H to give peaks at m/z 127, 101 and m/z 76, respectively.

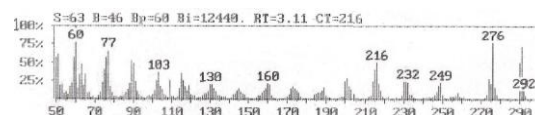
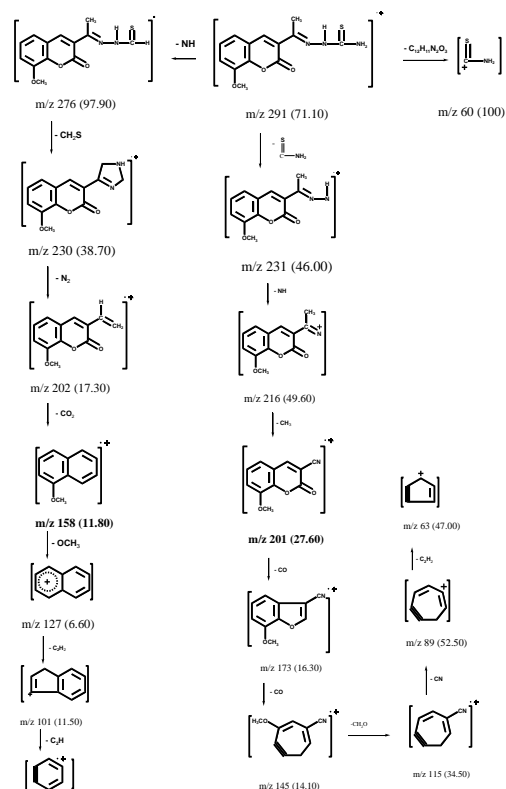


Figure 2. Mass Spectral Fragmentation pattern of compound 3



Scheme 3. Main Fragmentation pathway of compound 3

The molecular ion of compound **3** was also found to undergo fragmentation to produce the common peak at m/z 60 and the fragment ion of m/z 231, corresponding to the 1-(8-methoxycoumarin-3-yl) ethylidenehydrazino cation radical.

The fragment ion at m/z 231 broke to give an ion at m/z 216 which lost NH. The loss of methyl group (CH_3) and carbon monoxide from the ion with m/z 216 gave ions at m/z 201 and m/z 173, respectively. This fragmentation led to the fragments at m/z 145, 115, 89 and m/z 63, respectively.

The mass spectra of compounds **4(a)** and **4(b)** are fully consistent with assigned structures. In most cases, intense molecular ion peaks were observed. Thus, compounds **4(a)** and **4(b)** showed intense molecular ion peaks at m/z 405 and m/z 421, consistent with molecular formula $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_3\text{S}$ and $\text{C}_{22}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$, respectively.

The molecular ion of compound **4(a)** (Figure 3) underwent fragmentation to produce peak at m/z 189, corresponding to 5-(4-methyl-phenyl)-2-aminothiazole radical cation. It further underwent loss of NHCN , S, C_2H and C_2H_2 to give peaks at m/z 148, 116, 91 and m/z 65, respectively.

The molecular ion of compound **4(a)** was also found undergo fragmentation to produce ion at m/z 216, (Scheme 4) corresponding to 1-(8-methoxycoumarin-3-yl) ethylideneamino cation radical, which further broke to give an ion at m/z 200. The ion of m/z 200 broke to give an ion at m/z 174 which lost cyano

group (CN). Ion of m/z 174 fragmented to give ions at m/z 146 and m/z 116 which lost carbon monoxide and formaldehyde molecules.

The molecular ion of compound **4(a)** underwent fragmentation with rearrangement to produce a stable peak at m/z 271. The base ion of m/z 271 underwent loss of CH_2CN , CH_2CO and $\text{N}=\text{C}=\text{N}$, to give peaks at m/z 231, 188 and m/z 148, respectively. The ion of m/z 148 underwent loss of thioformyl group and acetylene molecule to give peaks at m/z 103 and m/z 77.

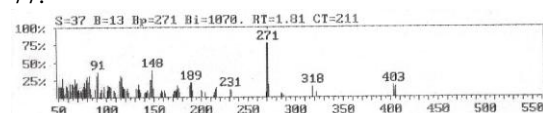
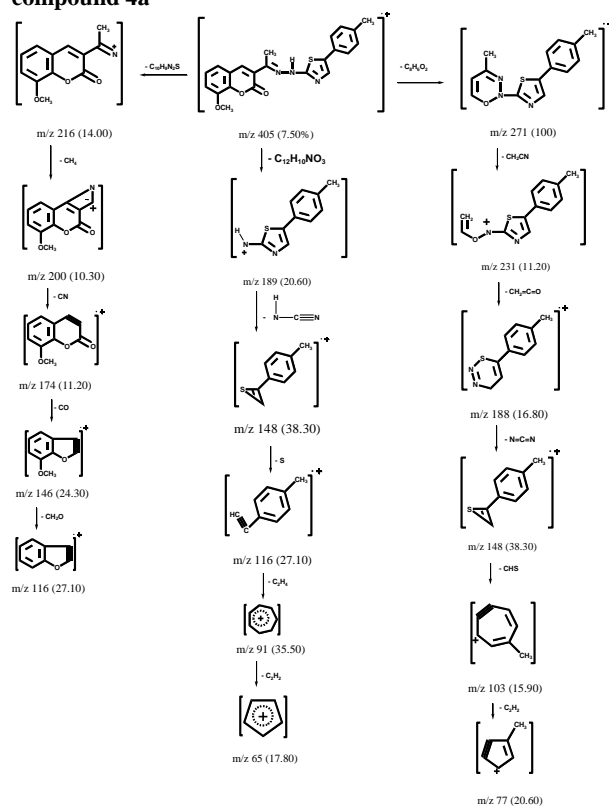


Figure 3. Mass Spectral Fragmentation pattern of compound 4a



Scheme 4. Main Fragmentation pathway of compound 4a

The mass spectra of compounds **5(a)** and **5(b)** show relatively small molecular ions and peaks typical of a cleavage and rearrangement processes type fragmentation. From the study of the mass spectra of compound **5(b)** (Figure 4), it was found that the molecular ion of compound fragmented further involved two various pathways as illustrated by Scheme 5.

The molecular ion of compound **5(b)** underwent fragmentation and rearrangement to produce a stable peak at m/z 318. This fragmentation led to the ion of m/z 276, 247, 206, 190, 164, 132 and m/z 102, respectively. Also the molecular ion of m/z 463

underwent loss of 5-(4-methoxyphenyl)-thiazole cation to give peak at m/z 231, which further broke to give an ion at m/z 216. It further underwent loss of CH_3 , HCN , CH_2O and CO to give peaks at m/z 201, 174, 144 and m/z 116, respectively.

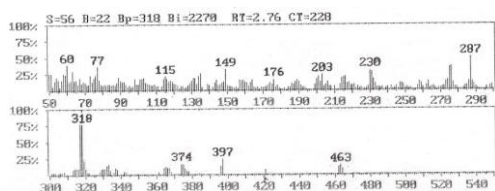
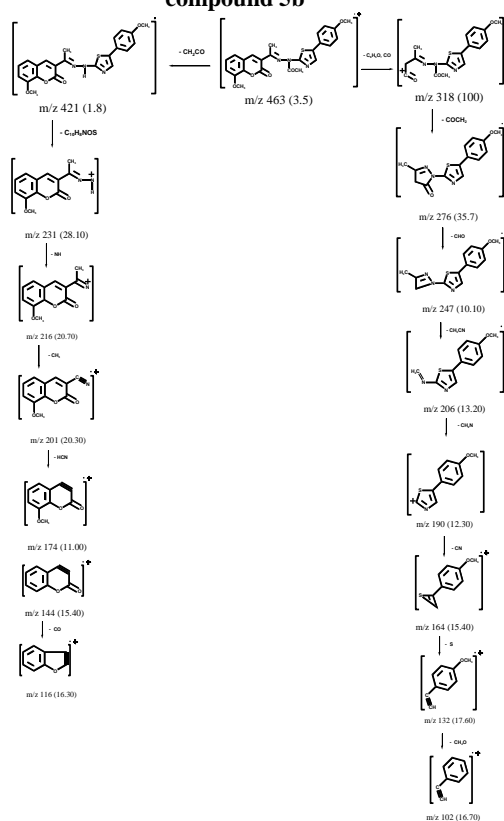


Figure 4. Mass Spectral Fragmentation pattern of compound 5b



Scheme 5. Main fragmentation pathway of compound 5b

Antimicrobial activity of the synthesized compounds is given in Table 1.

It is apparent from the data listed in Table 1, that some of the synthesized compounds showed antibacterial activity. Concerning the activity against Gram-positive bacteria, *B. subtilis*, compounds **4(b)** and **5(b)** showed moderate activity. While compounds **(2)**, **4(a)** and **6(a)** showed mild activity. Compounds **4(a, b)** and **5(b)** showed moderate activity against *S. penumonia*. Also, compound **5(b)** displayed moderate activity against *S. aureas*. On the other hand, the Gram-negative bacteria, *P. solanarium*, showed high responses to compound **4(a)**, while, compound **4(b)** showed moderate activity. Also, compounds **2**, **4b**, **5b**, **6a** showed

moderate activity against *E. coli*. From the data of antifungal activity, it is observed that compounds **4(b)**, **5(b)** are highly active against *A. niger*. Meanwhile, compound **4a** displayed moderate activity. Compounds **(2)** and **6(a)** showed mild activity. On the other hand, compound **4(b)** showed very good activity against *Penicillium sp.* Otherwise, compounds **4(a)** and **5(b)** displayed moderate activity.

Table 1. Biological activity of the prepared compounds

| Compound | | 2 | 4a | 4b | 5b | 6a | |
|--------------------------------|------------------------|---------------------|----|----|----|----|---|
| Organism / Relative Inhibition | Gram Positive Bacteria | <i>B. subtilis</i> | + | + | ++ | ++ | + |
| | | <i>S. penumonia</i> | - | ++ | ++ | ++ | - |
| | | <i>S. aureas</i> | - | + | + | ++ | + |
| Gram Negative Bacteria | <i>E. coli</i> | + | ++ | + | + | + | |
| | <i>P. sp.</i> | - | ++ | ++ | + | + | |
| | Antifungal Activity | <i>A. niger</i> | + | ++ | ++ | ++ | + |
| <i>Penicillium sp.</i> | | - | ++ | ++ | + | - | |

Note: (-)No antimicrobial activity, (+) Mild activity, (++) Moderate activity, (+++) very good activity.

Evaluation of Cellular Cytotoxicity:

The cytotoxic activity of compounds **5(b)** and **7(b)** against breast adenocarcinoma (MCF-7) cells was

determined using Doxorubicin (DOX) sulphorhodamine-B assay as a reference drug control [20, 21]. Each cell line was incubated with four concentrations (5-50 $\mu\text{g/ml}$) for each compound and was used to create compound concentration versus survival fraction curves. The response parameter Median Inhibition Concentration (IC_{50}) was calculated for each cell line (Table 2, Figures 5-7). The IC_{50} value corresponds to the compound's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 hrs). The antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor compounds and tumor selective agents [22]. In present study, the active analogs showed a distinctive potential pattern of selectivity as well as broad-spectrum antitumor activity. With regard to selectivity against individual cell lines, most of the compounds showed effectiveness against cell lines human breast cancer MCF-7 with IC_{50} values range of 12.5-13 $\mu\text{g/ml}$ comparative to DOX IC_{50} (2.97 $\mu\text{g/ml}$) (Table 2).

The activity of the tested compounds could be correlated to structure variation and modifications. All of the compounds showed high inhibition for the breast cancer cell line (MCF-7). This great inhibition at the mention concentration indicates a great potency for the compound with a strong lethal effect over (MCF-7) breast cancer cells.

Briefly the obtained screening results showed that, compounds **5(b)** and **7(b)** are the most active members with IC_{50} values 12.5-13 $\mu\text{g/ml}$. In case of compound **5(b)** the presence of acetyl, methoxy and thiazole ring moieties favored the antitumor activity against breast cancer MCF-7. In addition, an increase in antitumor activity over breast cancer was observed when the imidazolidinone thione moiety which contains -NH, -OH, and carbonyl groups in compound **7(b)** (IC_{50} values; 13 $\mu\text{g/ml}$) was attached to compound **7(b)** [18].

Table 2. *In vitro* antitumor activity of the 3-methoxy-2-hydroxybenzaldehyde derivatives

| Compound No. | IC_{50} ($\mu\text{g/ml}$) ^a |
|--------------|--|
| | MCF-7 ^b |
| 5b | 12.5 |
| 7b | 13 |
| DOX | 2.97 |

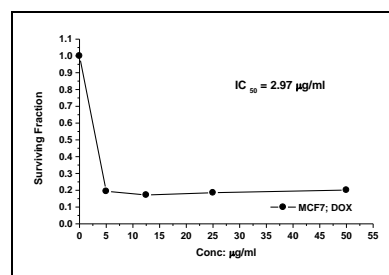


Figure 5. Growth inhibition of DOX on breast cancer cell lines MCF-7

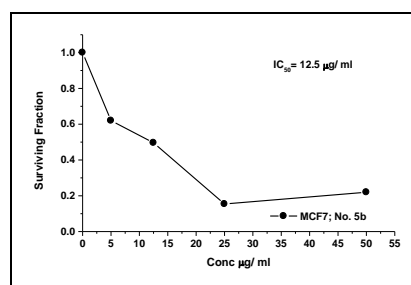


Figure 6. Growth inhibition of compound 5b on breast cancer cell lines MCF-7

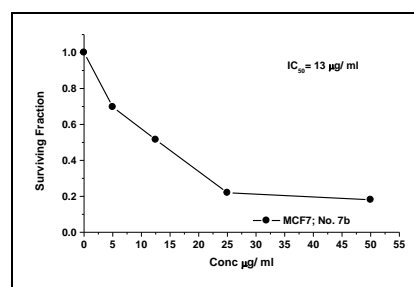


Figure 7. Growth inhibition of compound 7b on breast cancer cell lines MCF-7

"4. Conclusion"

Some 3-methoxy-2-hydroxybenzaldehyde derivatives have been synthesized. The mass spectral fragmentation patterns of prepared compounds have been investigated. *In vitro* antimicrobial activities of all synthesized compounds have been evaluated against five strains of bacterial culture and two fungi. Almost compounds show very good activity. All tested compounds showed high activity against human breast carcinoma cell line (MCF-7), with Median Inhibition Concentration (IC_{50}) range of 12.5-13 $\mu\text{g/ml}$.

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