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Research Article
Synthesis, Characterization, Biological Activity of 4-Oxo-imidazolidin-2-thione Derivatives

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Abstract

Backgrounds and Objective: This study discovers a new class coumarins that can be beneficial for antimicrobial and antioxidant agents, starting from 8-Methoxy-3-acetylcoumarin thiosemicarbazone 2. A series of some new heterocyclic compounds (3-8) incorporating coumarin moiety were synthesized and assessed for their antimicrobial activity and cytotoxic activity. The mass spectral fragmentation patterns of prepared compounds have been investigated in order to elucidate the structure of the synthesized compounds. **Materials and Methods:** *In vitro* antimicrobial activity of all synthesized compounds have been evaluated against five strains of bacterial culture, which includes three Gram +ve bacterial culture and two Gram -ve bacterial culture and two fungus. The potential cytotoxicity of the compounds (3-6) against MCF-7 cells was determined using Sulforhodamine-B assay. **Results:** Maximum 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). Compounds 5 and 6 are most active members with median inhibition concentration (IC_{50}) 9.45 and 11.1 μg mL^{-1}, respectively. **Conclusion:** All tested compounds showed high activity against human breast carcinoma cell line (MCF-7).

Key words: 4-Oxo-imidazolidin-2-thione derivatives, coumarin derivatives, knoevenagel condensation, breast cancer, mass spectral, cytotoxic activity

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hydantoins, a class of cyclic imides, have been demonstrated to possess good anticonvulsant properties[1-5]. Depending on the nature of substitution on the hydantoin ring, a wide range of the other pharmacological properties, e.g., fungicidial[6-8], herbicidal[9], antitumor[8,10], anti-HIV[11,] anti-inflammatory[12], hypblipienic[13] and antihypertensive activity[13], are also displayed. The coumarin derivatives are quite interesting objects for both synthesis and pharmacological screening. Their reactivity towards nucleophiles provides a useful route to prepare a variety of rearranged products and new heterocyclic systems[14]. There are many coumarin derivatives which have been reported for anticoagulant, antioxidant, anti-allergic, anti-cancer, anti-diabetic, anti proliferative and antiviral activities[12,15]. They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, dispersed fluorescent and laser dyes, insecticides and in optical brighteners[16,17]. In view of this 3-[1-(8-Methoxycoumarin-3-yl)ethylidene amino]-4-oxo-imidazolidin-2-thione 3 was prepared from the reaction of 8-Methoxy-3-acetyl-coumarin 1 with thiosemicarbazide to give 8-Methoxy-3-acetylcoumarin thiosemicarbazone 2, followed by the cyclization of 2 with ethyl chloroacetate in presence of fused sodium acetate. The chemical behavior of compound 3 towards acetic anhydride, phenyl diazonium chloride, 4-Methoxyphenacyl bromide and aromatic aldehydes was described. The electron impact (EI) ionization mass spectral fragmentation of some synthesized compounds was also described.

MATERIALS AND METHODS

This study have started since 2012. NMR spectra were recorded on a General Electric QE 300 instrument and chemical shifts are given in δ ppm^{-1} with TMS as internal references. IR spectra were recorded on a Perkin-Elmer 1420 and a Biorad FTS7 spectrometer in KBr peuets. Mass spectra were obtained on a Jeol JMS D-300 spectrometer operating at 70 eV. Microanalysis were conducted using a 1106 elemental analyzer. Melting points were determined on a Reichet hot instrument.

8-Methoxy-3-acetylcoumarin thiosemicarbazone (2): A mixture of 1 (0.01 mole), thiosemicarbazide (0.01 mole) and acetic acid (5 mL) in methanol (30 mL) was heated under reflux for 2 h, then cooled. The solid formed was filtered, washed with methanol, dried and purified by recrystalization from ethanol to give 2 as yellow crystals, yield 86%, melting point (m.p.) 205°C, IR (KBr): 3414, 3150 (NH_2), 3232 (NH), 1720 (C=O), 1618 (C=N), 1605, 1518 (C=C), 1325 (C=S), 1120, 1085 (C-O) cm^{-1}.

^{1}H-NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.29-7.33 (m, 3H, Ar-H), 7.97 (s, 2H, NH_2), 8.46 (s, 1H, H-pyrone), 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 (97.9), 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 (25.30), 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 (65.20), 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. $C_{13}H_{13}N_3O_3S$ requires: C, 53.61; H, 4.46; N, 14.43; S, 10.99.

3-[1-(8-Methoxycoumarin-3-yl)ethylidineamino]-4-oxo-imidazolidin-2-thione (3): A mixture of 2 (0.01 mole), ethylchloroacetate (0.01 mole) in acetic acid (30 mL) in presence of fused sodium acetate (0.03 mole) was heated under reflux for 3 h, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and purified by recrystallization from butanol to give 3 as yellow crystals, yield 78%, melting point 232°C. IR (KBr): 3289 (NH), 1720, 1705 (C=O), 1621 (C=N), 1317 (C=S), 1225, 1089 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 3.34 (s, 2H, $NHCH_2CO$), 3.92 (s, 3H, OCH_3), 7.31-7.42 (m, 3H,Ar-H), 8.16 (s, 1H, H-pyrone), 11.03 (s, 1H, NH) ppm. Anal. Found: C, 54.18; H, 3.63; N, 12.46; S, 9.51. $C_{15}H_{13}N_3O_4S$ requires: C, 54.38; H, 3.93; N, 12.69; S, 9.67.

1-Acetyl-3-[1-(8-methoxycoumarin-3-yl)ethylideneamino]-4-oxo-imidazolidin-2-thione (4): A solution of 3 (0.01 mole) in acetic anhydride (20 mL) was heated under reflux for 2 h, then cooled and poured into ice-water. The solid obtained was filtered off, washed with water, dried and purified by recrystallization from ethanol to give 4 as pale yellow crystals, yield 63%, m.p. 260°C. IR (KBr): 1723, 1705, 1691 (C=O), 1621

(C=N), 1605, 1592 (C=C), 1318 (C=S), 1215, 1120, 1083 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 3.35 (s, 3H, $COCH_3$), 3.91 (s, 3H, OCH_3), 4.35 (s, 2H, NCH_2CO), 7.38-7.45 (m, 3H, Ar-H), 8.56 (s, 1H, H-pyrane) ppm. Anal. Found: C, 54.53; H, 4.01; N, 11.03; S, 8.38. $C_{17}H_{15}N_3O_5S$ requires: C, 54.69; H, 4.02; N, 11.26; S, 8.58.

1-(4-Methoxybenzoyl) methyl-3-[1-(8-methoxycoumarin-3-yl)ethylideneamino]-4-oxo-imidazolidin-2-thione (5): A mixture of 3 (0.01 mole), 4-Methoxyphenacyl bromide (0.01 mole) and fused sodium acetate (0.05 mole) in acetic acid (40 mL) was heated under reflux for 6 h, then cooled and poured into water. The solid formed was filtered off, washed with water, dried and purified by recrystallization from methanol to give 5 as yellow crystals, yield 68%, m.p. 145°C. IR (KBr): 1723-1689 (br., CO), 1622 (C=N), 1610, 1589 (C=C), 1318 (C=S) cm^{-1}, 1225, 1121, 1080 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 3.36 (s, 2H, NCH_2CO), 4.16 (s, 2H, NCH_2CO), 3.85-3.98 (s, 6H, 2xOCH_3), 7.12-7.38 (m, 3H, Ar-H), 8.29 (s, 1H, H-pyrane) ppm. Anal. Found: C, 60.02; H, 4.13; N, 8.62; S, 6.48. $C_{24}H_{21}N_3O_6S$ requires: C, 60.12; H, 4.38; N, 8.77; S, 6.68.

3-[1-(8-Methoxycoumarin-3-yl)ethylideneamino]-4-oxo-5-phenylazo-imidazoliden-2-thione (6): A solution of 3(0.01 mole) in aqueous sodium hydroxide (50 mL, 10%) was chilled in ice 100-5°C. A cold aqueous solution (0-5°C) of the phenyl diazonium chloride (0.02 mole) was added dropwise with stirring during 45 min. After addition the mixture was stirred for further 30 min and then left for 2 h in a refrigerator. The precipitated product was collected, washed water, dried and purified by recrystallization with ethanol to give 6 as orange crystals, yield 62%, m.p. 168°C. IR (KBr): 3295 (NH), 1723 (C=O), 1702 (C=O), 1621 (C=N), 1611, 1605, 1581 (C=C), 1317 (C=S), 1212, 1083 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.11-7.72 (m, 9H, Ar-H and H-imidazole), 8.52 (s, 1H, H-pyrane) , 10.65 (s, 1H, NH) ppm. Anal. Found: C, 57.63; H, 3.66; N, 15.98; S, 7.13. $C_{21}H_{17}N_5O_4S$ requires: C, 57.93; H, 3.91; N, 16.09; S, 7.35.

5-Arylidene-3-[1-(8-methoxycoumarin-3-yl)ethylidene amino]-4-oxo-imidazolidin-2-thiones (7a,b): A mixture of 3 (0.01 mole), aromatic aldehydes (such as benzaldehyde and 2-hydroxybenzaldehyde, 0.01 mole) and piperidine (1 mL) was fused on a hot plate at 120-125°C for 1 h. The reaction mixture was cooled and acidified with dilute hydrochloric acid (2N). The crude product was filtered off, washed with water, dried and purified by recrystallization from ethanol to give 7.

3-[1-(8-Methoxycoumarin-3-yl)ethylideneamino]-4-oxo-5-benzylidene-imidazoliden-2-thione (7a): As yellow crystals, yield 71%, m.p. 261°C. IR (KBr): 3225 (NH), 1721, 1703 (C=O), 1622 (C=N), 1612, 1605, 1583 (C=C), 1319 (C=S), 1212, 1891, 1071 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 7.21-7.43 (m, 9H, Ar-H and H-olefinic), 8.53 (s, 1H, H-pyrane), 10.58 (s, 1H, NH) ppm. Anal. Found: C, 62.93; H, 4.01; N, 9.99; S, 7.46. $C_{22}H_{17}N_3O_4S$ requires: C, 63.01; H, 4.06; N, 10.02; S, 7.64.

3-[1-(8-Methoxycoumarin-3-yl)ethylideneamino]-4-oxo-5-(2-hydroxy)benzylidene-imidazoliden-2-thione (7b): As yellow crystals, yield 62%, m.p. 261°C. IR (KBr): 3229 (NH), 3350-3080 (br. OH), 1725, 1704 (C=O), 1621 (C=N), 1605, 1589 (C=C), 1319 (C=S), 1218, 1171, 1085(C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.58 (s, 3H, CH_3), 3.92 (s, 3H, OCH3), 7.31-7.50 (m, 8H, Ar-H and H-olefinic), 8.63 (s, 1H, H-pyrane), 10.68 (s, 1H, NH) and 11.60 (s, 1H, OH) ppm. MS: m/z (%)= 437 (M^++2, 21.9), 436 (M^++1, 18.8), 435 (M^+, 18.8), 367 (21.9), 366 (18.8), 356 (18.8), 355 (18.8), 329 (6.3), 328 (21.9), 286 (21.9), 285 (12.5), 283 (15.6), 282 (15.6), 262 (18.8), 261 (9.4), 254 (9.4), 253 (25.0), 220 (18.8), 219 (25.0), 202 (93.8), 199 (18.8), 198 (18.8), 197 (18.8), 191 (18.8), 190 (25.0), 177 (31.3), 176 (28.1), 175 (50.0), 171 (18.8), 170 (28.1), 169 (15.6), 162 (18.8), 161 (18.8), 160 (25.0), 140 (15.6), 139 (15.6), 133 (37.5), 132 (12.5), 131 (21.9), 127 (21.9), 120 (28.1), 118 (28.1), 117 (25.0), 116 (21.9), 105 (34.4), 104 (31.3), 97 (15.6), 96 (18.8), 95 (28.1), 92 (21.9), 90 (28.1), 89 (37.5), 85 (25.0), 84 (18.8), 82 (21.9), 77 (53.0), 76 (40.6), 75 (34.4), 74 (31.3), 71 (9.4), 68 (31.3), 67 (18.8), 66 21.9), 65 (21.9), 64 (31.3), 63 (34.3), 62 (28.1), 60 (31.3), 59 (28.1), 57 (37.5), 56 (31.3), 55 (37.5), 52 (34.4), 51 (53.1), 50 (62.5). Anal. Found: C, 60.48; H, 3.79; N, 9.49; S, 7.21. $C_{22}H_{17}N_3O_5S$ requires: C, 60.69; H, 3.91; N, 9.65; S, 7.36.

1-Acetyl-3-[1-(8-Methoxycoumarin-3-yl)ethylideneamino]-4-oxo-5-benzylidene-imidazolidin-2-thione (8): A solution of 7a (0.01 mole) in acetic anhydride (20 mL) was heated under reflux for 2 h, then cooled and poured into ice-water. The resulting product was filtered, washed with water, dried and purified by recrystallization from benzene to give 8 as pale yellow crystals, yield 53%, m.p. 155°C. IR (KBr): 1722-1698 (br. C=O), 1621 (C=N), 1608, 1598 (C=C), 1318 (C=S), 1215, 1171, 1075 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.29 (s, 3H, CH_3), 2.50 (s, 3H, $COCH_3$), 3.89 (s, 3H, OCH_3), 7.36-7.44 (m, 9H, Ar-H and H-olefinic), 8.56 (s, 1H, H-pyrone) ppm. Anal. Found: C, 62.27; H, 4.03; N, 9.02; S, 6.79. $C_{24}H_{19}N_3O_5S$ requires: C, 62.47; H, 4.12; N, 9.11; S, 6.94.

Table 1: Biological activity of the prepared compounds

	Organism/relative Inhibition						
	Gram positive bacteria			Gram negative bacteria		Antifungal activity	
Compounds	*Bacillus subtilis*	*Streptococcus penumonia*	*Staphylococcus aureas*	*Escherichia coli*	*Pseudomonas* sp.	*Aspergillus niger*	*Penicillium* sp.
1	+	-	-	+	-	+	-
3	+	-	+	+	+	+	-
4	++	+	+	+	+	+	++
5	+++	++	++	++	+	++	++
6	++	+++	+++	+++	+	+++	+++
7a	++	+	+	++	++	+	+
7b	+++	+++	+++	+	+++	+++	++
8a	+	++	++	++	+	+	+

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

Biological evaluation

Antimicrobial activity: The antimicrobial activity was assayed by cup plate agar diffusion method, Vyas *et al.*[18] and Lakshminarayanan *et al.*[19] 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, 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.

Antibacterial activity: The purified products were screened for their antibacterial activity by using cup-plate agar diffusion method[8]. The nutrient agar broth prepared by the usual method, was inoculated aseptically with 0.5 mL of 24 h old subculture of *Bacillus subtilis, Streptococcus penumonia, Staphylococcus aureas, Escherichia coli* and *Pseudomonas 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 2 h. 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^{-1}) solution of sample in DMF. The plates were incubated at 37°C for 24 h 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 and recorded in Table 1.

Antifungal activity: *Aspergillus niger* and *Penicillium* sp. were employed for testing antifungal activity by cup-plate agar diffusion method[8,20,21]. The culture was maintained on subrouse dextrose agar slants. Sterilized sub rouse dextrose agar medium was inoculated with 72 h 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 h. 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 inculated at 30°C for 48 h. 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. The zones of inhibition are recorded in Table 1.

Measurement of potential cytotoxicity by SRB assay: The potential cytotoxicity of the compounds (3-6) against MCF-7 cells was determined using Sulforhodamine-B assay[22].

RESULTS

Chemistry: 8-Methoxy-3-acetylcoumarin 1 was prepared by the reaction of 3-Methoxy-2-hydroxy benzaldehyde with ethyl acetoacetate in presence of piperidine[8,17]. Condensation of 8-Methoxy-3-acetylcoumarin 1 with thiosemicarbazide in methanol in presence of acid medium gave the corresponding 8-Methoxy-3-acetylcoumarin thiosemicarbazone 2. Treatment of compound 2 with ethyl chloroacetate in presence of fused sodium acetate in acetic acid under reflux[17], yielded the corresponding 3-[1-(8-Methoxycoumarin-3-yl) ethylidene amino]-4-oxo-imidazolidin-2-thione 3, (Fig.1). Acetylation of 3-[1-(8-Methoxycoumarin-3-yl)ethylidene amino]-4-oxo-imidazolidin-2-thione with acetic anhydride under reflux led to the formation of 1-Acetyl-3[1-(8-Methoxycoumarin-3-yl)ethylidene amino]-4-oxo-imidazolidin-2-thione 4. Alkylation of compound 3 with 4-methoxyphenacyl bromide in presence of fused sodium acetate in acetic acid under reflux gave the corresponding 1-(4-Methoxybenzoylmethyl)-3-[1-(8-methoxycoumarin-3-yl) ethylidene amino]-4-oxo-imidazolidin-2-thione 5.

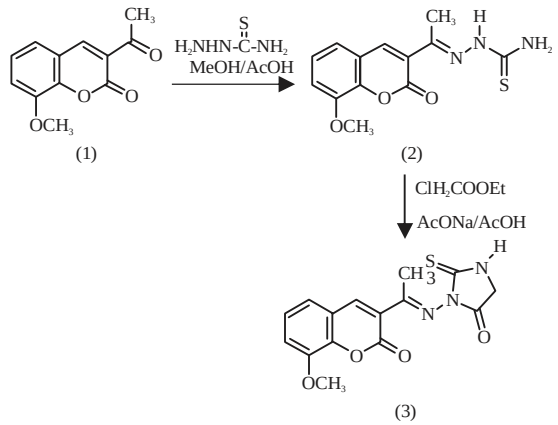

Fig. 1: Synthesis of compound 3

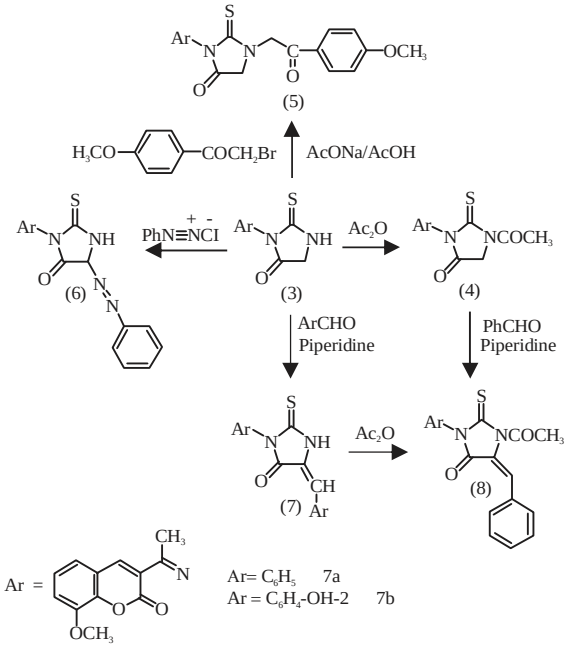

Fig. 2: Synthesis of compound 3 derivatives

Diazotization[23] of aniline followed by coupling with sodium salt of 3-[1-(8-Methoxycoumarin-3-yl) ethylidene amino]-4-oxo-imidazolidin-2-thione 3 gave the corresponding 5-Phenylazo-3-[1-(8-methoxycoumarin-3-yl) ethylideneamino]-4-oxo-imidazolidin-2-thione 6. Condensation[24] of compound 3 with aromatic aldehydes (such as benzaldehyde and 2-hydroxybenzaldehyde) in presence of piperidine under fusion led to the formation of 5-Arylidene-3-[1-(8-methoxycoumarin-3-yl) ethylidene amino]-4-oxo-imidazolidene-2-thione 7a,b. Acetylation of compound 7a with acetic anhydride led to the formation of 1-Acetyl-3-[1-(8-methoxycoumarin-3-yl) ethylidene amino]-4-oxo-5-benzylidene-imidazolidin-2-thione 8 (Fig. 2). The structure of 8 was also established via reaction of compound 4 with benzaldehyde in presence of piperidine.

Compounds 3 and 4: The mass spectra of the synthesized compounds 3 and 4 showed intense molecular ion peaks at m/z 331 and 373, consistent with the molecular formula $C_{15}H_{13}N_3O_4S$ and $C_{17}H_{15}N_3O_5S$, respectively (Fig.3, 4a, b).

Compounds 5 and 6: The mass spectra of compound 5 and 6 are fully consistent with assigned structures. In most cases,

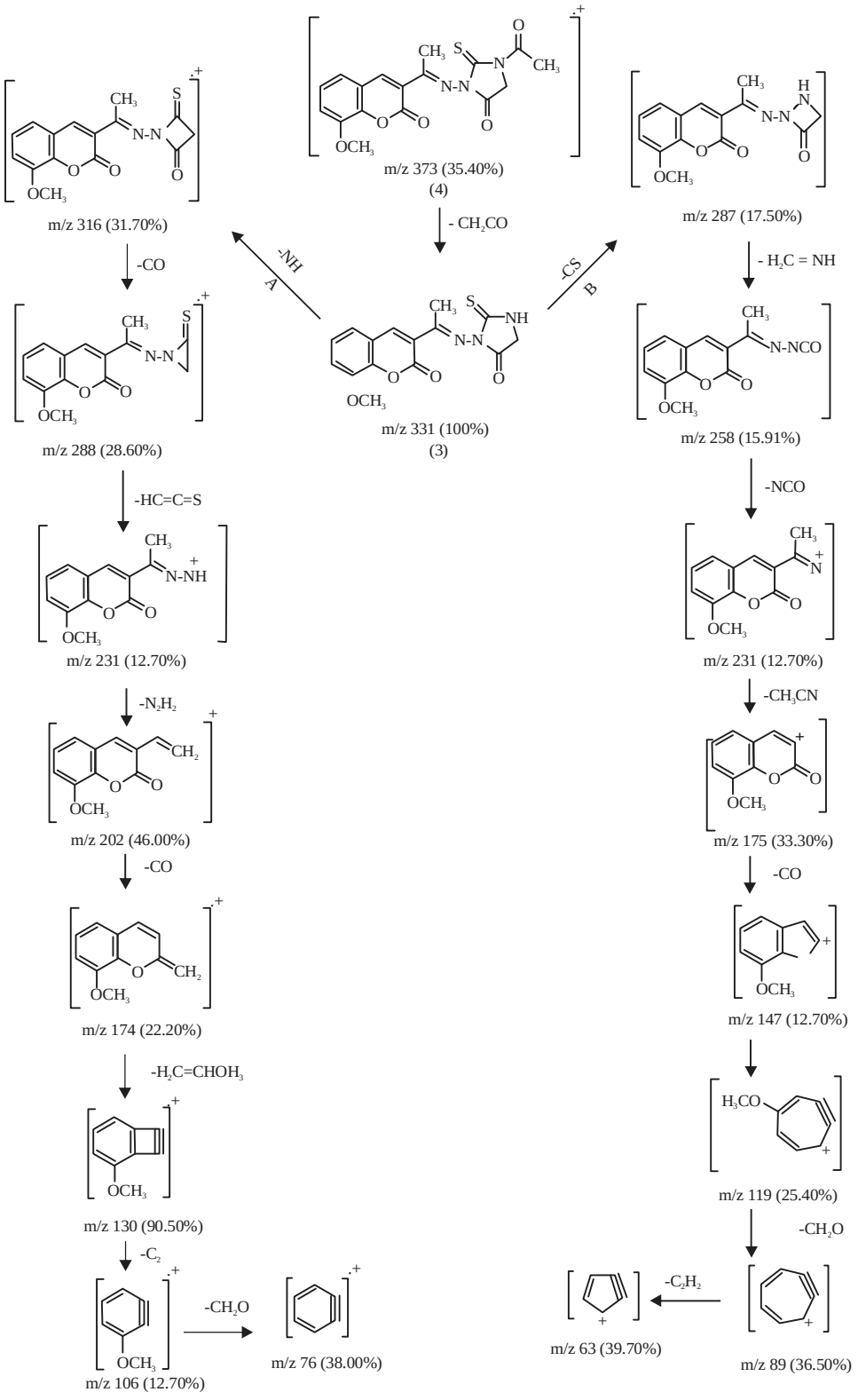

Fig. 3: Main fragmentation pathway of compounds 3 and 4

intense molecular ion peaks were observed. Thus, compound 5 and 6 showed an intense molecular ion peak at m/z 479 and 435, corresponding to the molecular formula $C_{24}H_{21}N_3O_6S$ and $C_{21}H_{17}N_5O_4S$, respectively (Fig. 5, 6a, b).

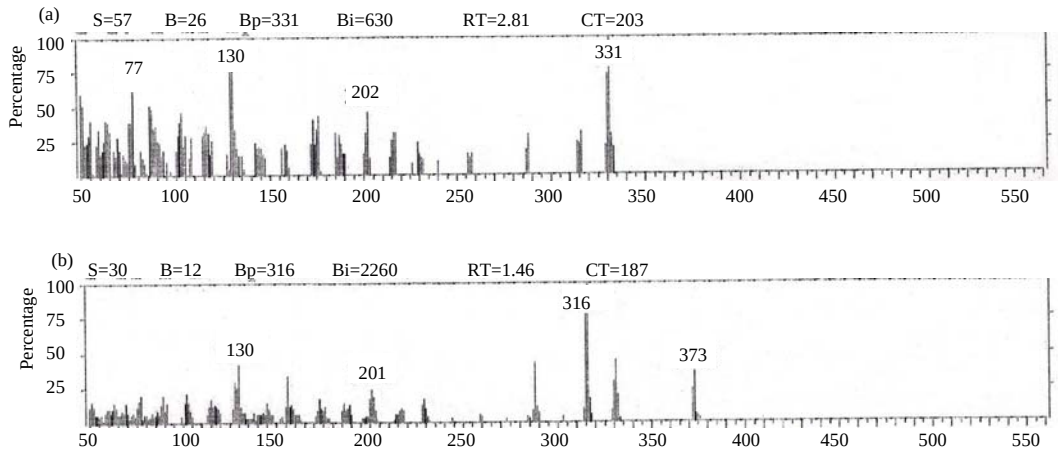

Fig. 4a-b: Mass spectral fragmentation pattern of (a) compound 3 and (b) compound 4

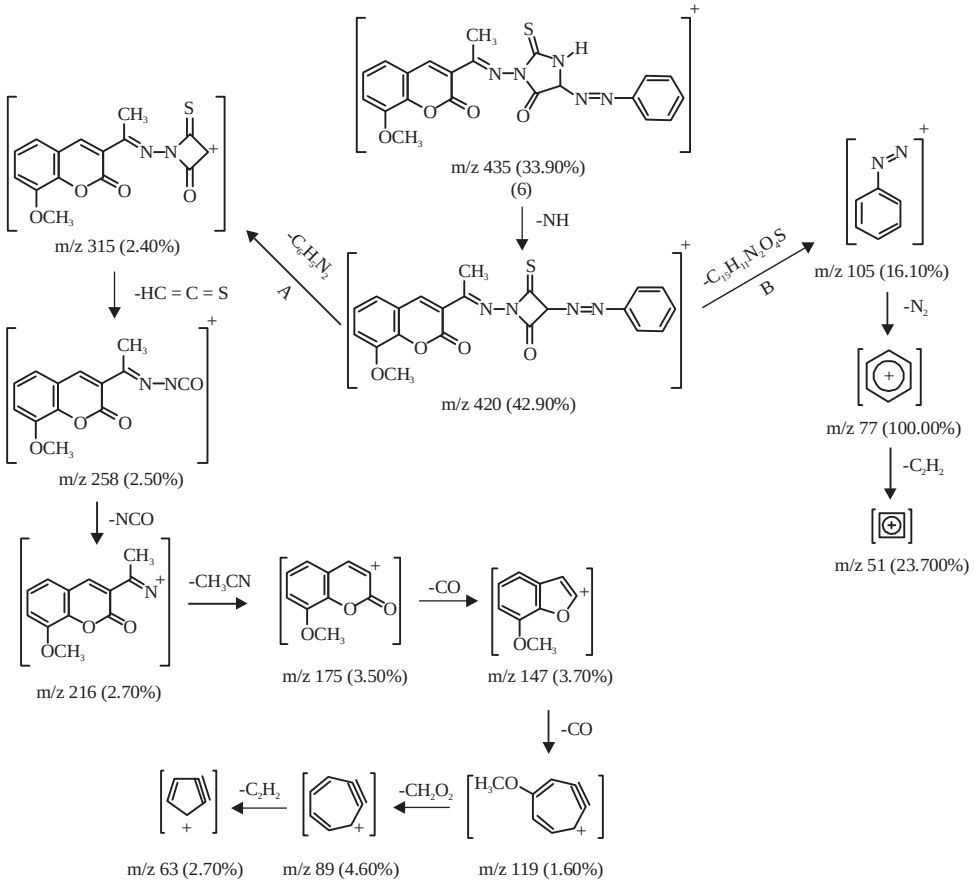

Fig. 5: Main fragmentation pathway of compound 6

Compounds 7 and 8: The mass sprctra of compounds 7 and 8, illustrated in Fig. 7a, b, respectively. It was found that the molecular ion fragmented further and involved two pathways as illustrated in Fig. 8.

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

Evaluation of cellular cytotoxicity: The cytotoxic activity of compounds 3-6 against breast adenocarcinoma (MCF-7) cells was determined using Doxorubicin (DOX) sulphorhodamine-B assay as a reference drug control[25,26]. Each cell line was incubated with four concentrations (5-50 μg mL^{-1}) for each compound and was used to create compound concentration

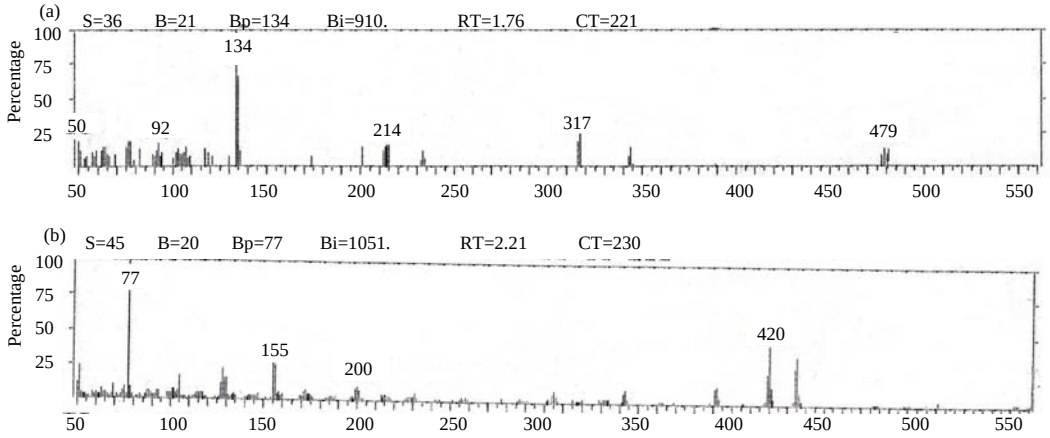

Fig. 6a-b: Mass spectral fragmentation pattern of (a) compound 5 and (b) compound 6

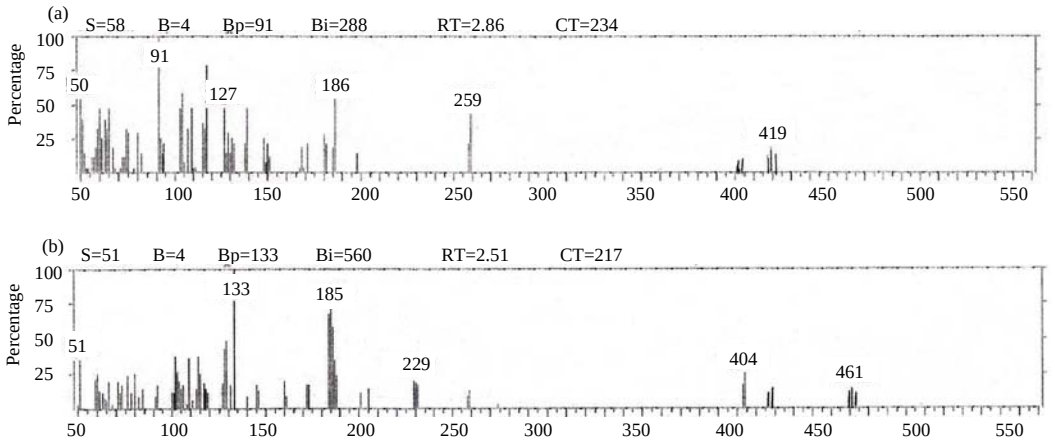

Fig. 7a-b: Mass spectral fragmentation pattern of (a) compound 7a and compound 8a

Table 2: *In vitro* antitumor activity of the 4-Oxo-imidazolidin-2-thione derivatives

Compound No.	(IC_{50}/μg mL^{-1})[a] MCF-7[b]
3	12.3
4	14
5	9.45
6	11.1
DOX	2.97

[a]IC_{50}, Compound concentration required to inhibit tumor cell proliferation by 50%, [b]Human breast cell line (MCF-7)

versus survival fraction curves. The response parameter (IC_{50}) was calculated for each cell line (Table 2). 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 h).The potential cytotoxicity of the compounds (3-6) against MCF-7 cells was demonstrated in (Table 2, Fig. 9a-e).

DISCUSSION

There is an imminent need to develop new anticancer drugs. The antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor compounds and tumor selective agents[27]. 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 9.45-14 μg mL^{-1} comparative to DOX IC_{50} (2.97 μg mL^{-1}) (Table 2, Fig. 9a-e). 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, among the tested compounds, compounds 5 and 6 are the most active members with IC_{50} values 9.45 and 11.1 μg mL^{-1}. In case of compound 5, the presence of 4-methoxy phenacyl moiety which contains methoxy group favoured the antitumor activity against breast

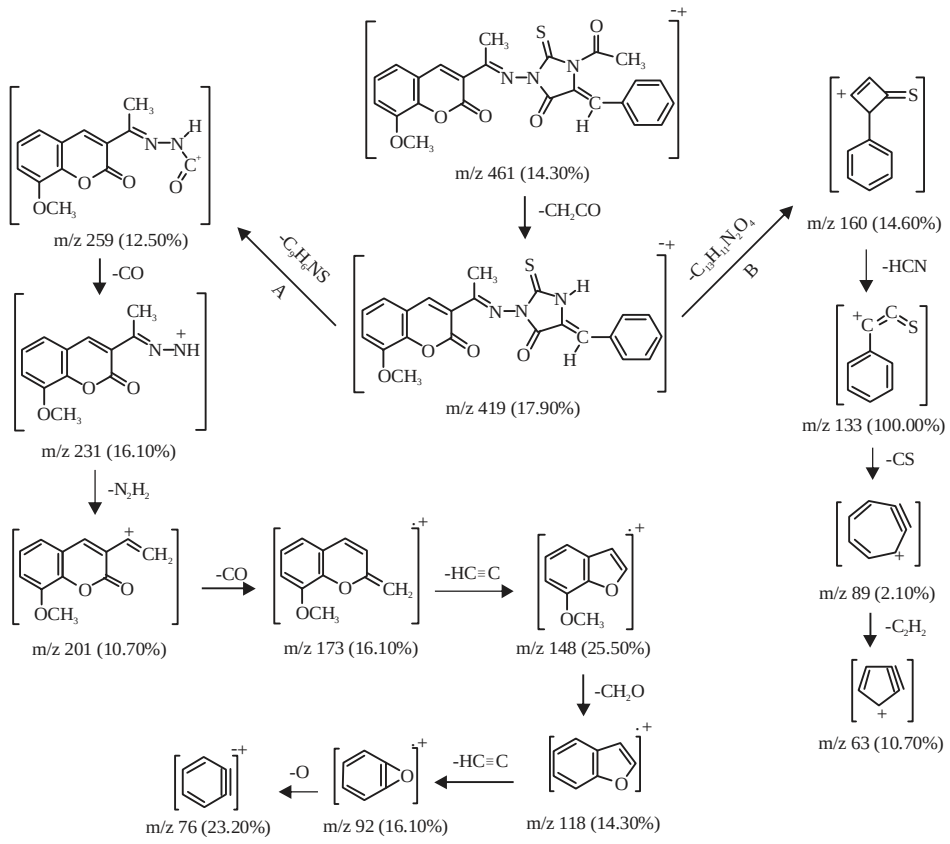

Fig. 8: Main fragmentation pathway of compounds 7a and 8

cancer MCF-7. While replacement of such group with phenyl azo- moiety in compound 6, lead to slightly decrease in cytotoxic activity against MCF-7 cell lines. In addition, an increase in antitumor activity over breast cancer was observed when the methoxy group (IC_{50} values, 11.1 µg mL^{-1}) was attached to compound 6[22].

In the present study, seven 4-oxo-imidazolidin-2-thione derivatives (3-8) have been synthesized. Compounds 5 and 6 are the most active analyzed members with Median Inhibition Concentration (IC_{50}) 9.45 and 11.1 µg mL^{-1}, respectively. Other studies on the 4-Oxo-imidazolidin-2-thione were prepared by El-Shenawy[28] 5-Phenyl-2-[(3,4,5-trimethoxybenzylidene) hydrazino]thiazole and 3-[(3,4,5-trimethoxybenzy lidene)amino]4-oxo-imidazolidin-2-thione were prepared. These compounds exhibited high antimicrobial and antifungal activities and compound 3-[(3,4,5-Trimethoxybenzylidene)amino]-5-(2-hydroxy)benzylidene-4-oxo-imidazolidin-2-thione being the most active one against colon carcinoma cell line (HCT-116). On the other hand, series of 2-Thioxo-imidazolidin-4-one derivatives containing benzylidene amino and phenylethylidene amino groups have been prepared by Elhady[29], among the tested compounds Aryl-[1-(arylidine) amino-2-thioxo-4-oxo-imidazolidin-3-yl)carbanols is found to has lowest IC_{50} value (3.79 µg). Another series of derivatives of compound 3-[(bezocoumarin-3-ylethylidene)amino]-4-oxo-imidazolidin-2-thione were also synthesized by El-Deen *et al.*[30]. Compounds 3-[(Benzocoumarin-3-ylethylidene) amino]-4-oxo-5-benzylidene-imidazolidin-2-thione and 1-Acetyl-3-[(benzocoumarin-3-ylethylidene)amino]-4-oxo-imidazolidin-2-thione are the most active compounds against MCF-7 cell lines with IC_{50} 14.97 and 19.48 µg mL^{-1}, respectively. So the structural rigidity imposed by the modification may have imparted a different biological activity to the molecule. Hence, this class of compounds might be potentially useful in the field of cancer treatment.

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, *Bacillus subtilis,* the compounds 6, 7b showed very good activity. While compounds 4, 6, 7a showed moderate activity and compounds 1, 3, 8a displayed mild activity. Meanwhile, compounds 6, 7b displayed good activity against *Streptococcus penumonia.* Otherwise, compounds 8a

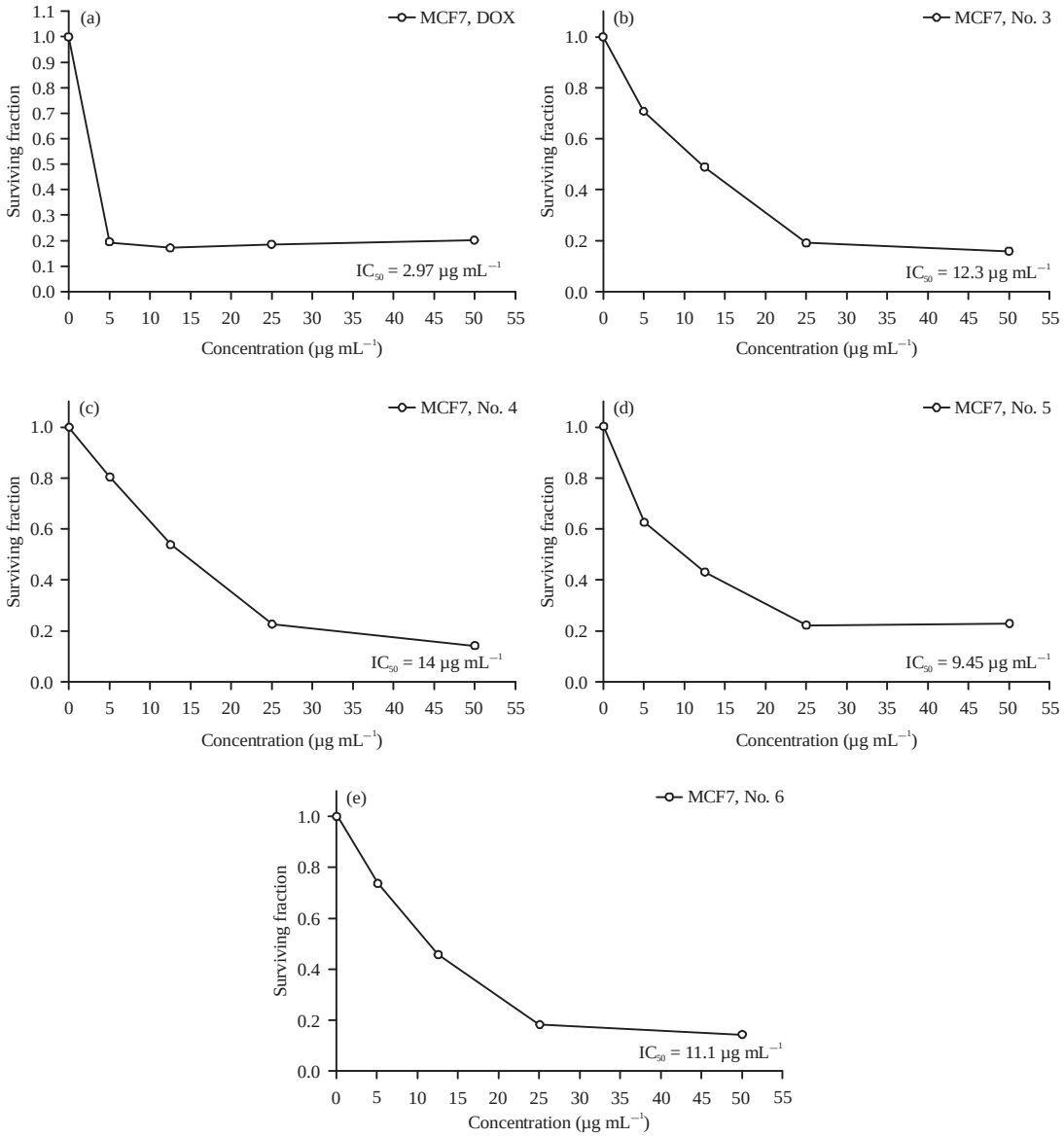

Fig. 9a:Growth inhibition of DOX on breast cancer cell lines (a) MCF-7, (b) Compound 3 on breast cancer cell lines MCF-7, (C) Compound 4 on breast cancer cell lines MCF-7, (d) Compound 5 on breast cancer cell lines MCF-7 and (e) Compound 6 on breast cancer cell lines MCF-7

showed moderate activity and compound 4, 7a exhibit mild activity. While. Componds 6, 7a exhibited very good activity against *Streptococcus penumonia* and *Staphylococcus aureas,* while compounds 5, 8a showed moderate activity. Otherwise, compounds 4, 7a displayed mild activity against *Streptococcus penumonia* and *Staphylococcus aureas.* However, compounds 4, 7a showed mild activity against them. Concerning the activity against Gram-negative bacteria, compounds 6 showed very good activity against *Escherichia coli,* also 7b showed very good activity against *Pseudomonas solanarium*. Meanwhile, 5, 7a, 8a exhibited moderate activity against *Escherichia coli.* Whereas, compound 3, 4, 5, 6, 7a, 8a showed moderate activity against *Pseudomonas solanarium*. However, 1, 3, 4, 7b showed mild activity against *Escherichia coli* and 3, 4, 5, 6, 7a, 8a showed mild activity against *Pseudomonas solanarium.* From the data of antifungal activity, it is observed that compound 6 showed very good activity against *Aspergillus niger* and *Penicillium* sp. Also, compound 7a showed very good activity against *Aspergillus niger*.

Meanwhile, compounds 4, 5, 7b displayed moderate activity against *Penicillium* sp. but compounds 7a, 8a exhibited mild activity against *Penicillium* sp. and compound 5 showed mild activity against *Aspergillus niger.*

The mass spectral decomposition modes[8,10,31] of various 2-Thioxo-imidazolidinones containing 1-(8-Methoxycoumarin-3-yl) ethylidene amino substituent have been investigated and fragmentation pathways have been suggested. From the study of the mass spectra of compound 3, it was found that the molecular ion for the compound fragmented further, a long two different pathways. The molecular ion of m/z 331 for compound 3 fragmented via pathway A (Fig. 3) gave the fragment of m/z 316 by losing NH group. The fragment of m/z 316, which fragmented to give the fragment of m/z 288 by losing carbon monoxide. The fragment of m/z 288 was broken to give an ion of m/z 231. This fragmentation led to fragments of m/z 202, 174, 130, 106 and m/z 76, respectively. Accordingly, the same molecular ion of m/z 331 fragmented via the pathway B to give the ion of m/z 287 by losing thiocarbon monoxide (C=S), which lost CH_2NH to give the ion of m/z 258. The fragment of m/z 258, which fragmented to give the fragment of m/z 216 by losing isocyanate group (NCO). The fragment of m/z 216 was broken to give an ion of m/z 175. This fragmentation led to fragments of m/z 147, 119, 89 and m/z 63 (Fig. 4a). From the mass spectrum of 4 (Fig. 4b), it was concluded that the molecular ion was at m/z 373. The ion of m/z 373 underwent fragmentation to produce a fragment at m/z 331 by losing ketone molecule (CH_2CO), corresponding to the molecular ion of compound 3. The fragment of m/z 331 further broke via pathway similar to compound 3 (Fig. 3). The electron impact ionization mass spectrum of compound 3 shows a base peak at m/z 331 (molecular ion), while the compound 4a base peak at m/z 316. The molecular ion of m/z 479 for compound 5 (Fig. 6a) fragmented further along two various suggested pathways as illustrated in Fig. 5. The molecular ion of m/z 435 for compound 6 (Fig. 6b) underwent fragmentation to produce a peak at m/z 420 by losing NH group. The ion of m/z 420 fragmented via pathway A to give the ion of m/z 315 by losing phenyl azo radical cation, which fragmented further to give the ion of m/z 258 by losing CH=C=S. The ion of m/z 258 was broken to give the ion of m/z 216 by losing isocyanate group (NCO). This fragmentation led to ion of m/z 175, 147, 119, 89 and m/z 63, respectively. Accordingly, the same ion of m/z 420 fragmented via the pathway B to give a fragmented ion of m/z 105. It further underwent loss of nitrogen (N_2) to give stable ion at m/z 77, which further fragmented and gave a fragment of m/z 51 by losing acetylene molecule.

The molecular ion of m/z 419 for compound 7 (Fig. 7a, 8) fragmented with rearrangement via the pathway A to give the ion of m/z 259, which fragmented further to give the ion of m/z 231 by losing carbon monoxide. The ion of m/z 231 was broken to give the ion of m/z 201 by losing N_2H_2 molecule (HN=NH). This fragmentation led to ion of m/z 173, 148, 118, 92 and m/z 76, respectively. Accordingly, the same molecular ion of m/z 419 fragmented via the pathway B to give a fragmented ion of m/z 160. It further loss of HCN, CS and acetylene molecule to give peaks at m/z 133, 89 and m/z 63, respectively. The molecular fragment of compound 8 (Fig. 7b, 8) (m/z 461) had fragmented to give the fragment of m/z 419, corresponding to the molecular ion of compound 7 by losing ketone molecule (CH_2CO). The fragment of m/z 419 was broken via pathway in the same fragmented processes which was observed for compound 7.

CONCLUSION

Seven 4-Oxo-imidazolidin-2-thione 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). Compounds 5 and 6 are most active members with Median Inhibition Concentration (IC_{50}) 9.45 and 11.1 μg mL^{-1}, respectively.

SIGNIFICANCE STATEMENTS

This study will help the researcher to uncover the critical areas of therapeutic importance of coumarins along with various methods of synthesis that many researchers were not able to explore. Thus a new class of antimicrobial and antioxidant agents, starting from 8-Methoxy-3-acetylcoumarin thiosemicarbazone 2, a series of some new heterocyclic compounds (3-8) incorporating coumarin moiety were synthesized. Such compounds were exploited in development of various important molecules which provide scaffolds for drug development.

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