Research Paper Science

Biological Activity and Mass Spectra Investigation of Some Coumarin Derivatives

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ABSTRACT

3-(Aroyl)amino-6,8-disubstituded coumarins(2a-c) were prepared via condensation of salicylaldehyde derivatives (1) with N-aroylglycine in presence of fused sodium acetate and acetic anhydride. Hydrolysis of 2a with hydrochloric acid 6N HCl yield the corresponding 3-amino-6-bromocoumarin(3), followed by condensation of 3 with 5-bromosalicylaldehyde to give 3-(5-bromo-2-hydroxybenzyliden)amino-6-bromo- coumarin(4).Acetylation and alkylation of 2b with acetic anhydride and ethylchloroacetate afforded the corresponding N-acetyl and N-alkyl derivatives(5and 6). EI mass spectrometric behavior of compounds 2a, b, 5 and 6 show a weak molecular ion peak and a base peak of 105, While the compound 2c a base peak of 139, resulting from a cleavage fragmentation. The compounds 3 and 4 give a characteristic fragmentation pattern with stable fragments of m/z 121 and m/z 226. Some representative compounds showed antimicrobial and antitumor activity in vitro by the drug diffusion methods.

Keywords :

Introduction

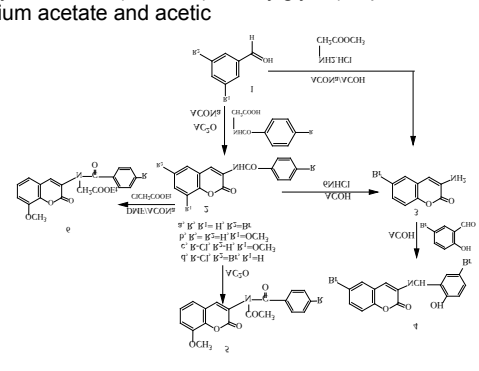
A review article dealing with the varied physiological activities of coumarin derivatives has been published, describing their anticoagulant, antimicrobial, antiheliminitic, hypothermal properties and vasodilaboratory action [1, 2].

During the last twenty years, the study of the biological activities of coumarin derivatives has been the aim of many researchers [4, 8]. Also, the structure activity relationships of coumarins have revealed that the presence of substituted thiocarbonyl mercaptoacetylamino derivatives is an essential feature of their pharmacological action. In continuation of our previous papers[7, 9] towards the synthesis of coumarin derivatives, we report an efficient preparation of 3, 6, 8,-trisubstituted coumarins starting from salicylaldehyde and N-aroyl glycin derivatives. The electron impact (EI) ionization mass spectral fragmentation of the prepared coumarin derivatives was described.

Synthesis

The synthetic pathways leading to the new coumarin derivatives are illustrated in (scheme 1).

3-(Aroyl)amino-6,8-disubstitutedcoumarin(**2a-c**) were prepared via cyclocondensa-tion of salicylaldehyde derivatives (namely, 5-bromo-2-hyroxybenzaldehyde and 3- methoxy-2-hydroxybenzaldehyde) with N-aroylglycin (such as, N-benzoyl glycine and N-(4-chloro)benzoylglycin) in presence of fused sodium acetate and acetic



anhydride under fusion. Hydrolysis of 3-(benzoyl)amino-6-bromocoumarin(**2a**)with hydrochloric acid(6N) in acetic acid under reflux led to the formation of 3-amino-6-bromocoumarin(**3**). Condensation of compound **3** with 5-bromo-2-hydroxy benzaldehyde in acetic acid yielded the corresponding 3-(5-bromo-2-hydroxy benzylidene)amino-6-bromocoumarin(**4**). The structure **4** was also established by treatment of 5-bromosalicyaldehyde (**1a**) with methylglycine ester hydrochloride in presence of fused sodium acetate in acetic acid under reflux.

Acetylation of 3-(benzoyl)amino-8-methoxy coumarin(**2b**) with acetic anhydride under reflux afforded the corresponding to 3-(N-acetyl-N-benzoyl)amino-8-methoxy coumarin (**5**).

Alkylation of 3-(benzoyl)amino-8-methoxycoumarin with ethylchloroacetate in dimethyl formamide in presence of fused sodium acetate yielded the corresponding to 3-(N-benzoyl-N-ethoxycarbonylmethyl)amino-8-methoxy coumarin (**6**).

Mass spectrometry

The mass spectral decomposition modes of various heterocyclic compounds containing coumarin substituents have been investigated and fragmentation pathways have been suggested.

Compounds 2a-c

The mass spectra of the synthesized compounds **2a**, **2b** and **2c** showed intense molecular ion peaks at m/z 343, 295 and 329, consistent with the molecular formula $C_{16}H_{10}NBrO_3$, $C_{17}H_{13}NO_4$ and $C_{17}H_{12}NClO_4$, respectively. From the study of mass spectra of compounds **2a-c** (Fig.1, 2, 3) it was found that the molecular ion for all these compounds fragmented further, along two different pathways, as illustrated by (scheme **2**). The molecular ion peaks at m/z 343, m/z 329, and m/z 295 for compounds **2a-c** fragmented via pathway A gave the fragment ions at m/z 238 and m/z 190 by losing substituents benzoyl cation. The fragmentations of m/z 238 and m/z 190, which broken to give peaks at m/z 223 and 175 by losing NH group. The fragmentations of m/z 223 and 175 were broken to give an ion at m/z 195 and 147. This fragmentation led to fragment ions at m/z 167, 119 and m/z 88, respectively.

According, the same molecular ions of m/z 329 and 295 fragmented via the pathway B to give the stable ions of m/z 105 and m/z 139, which lost carbon monoxide molecule to give the ions of m/z 77 and m/z 111. The loss of acetylene molecule from the fragments of m/z 77 and m/z 111, to give the fragment ions at m/z 51 and m/z 85.

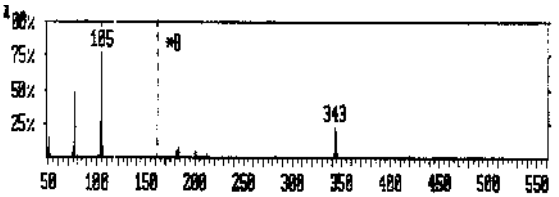


Fig. 1

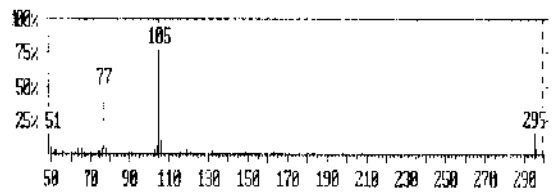


Fig. 2

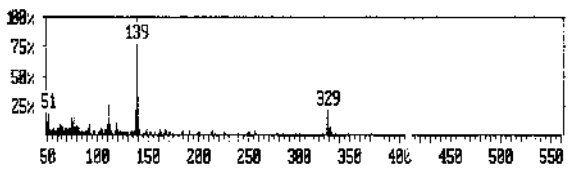
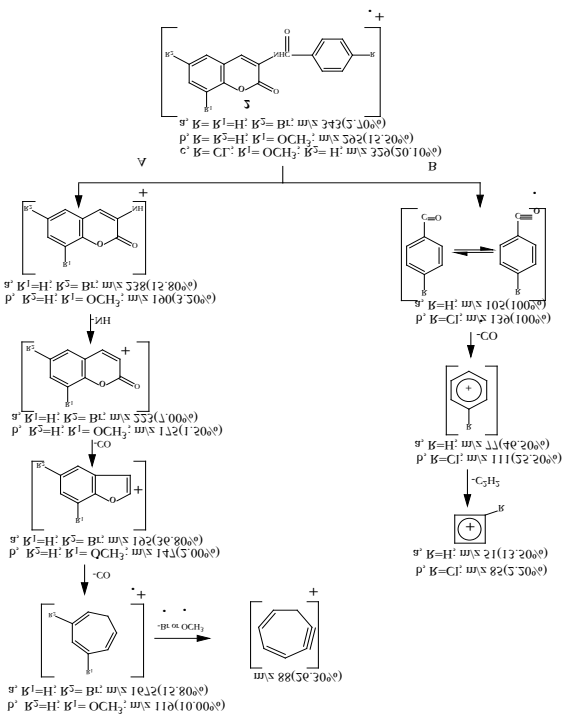


Fig. 3



Scheme 3: Main fragmentation pathways to compounds 3a-c.

Compound 4

The molecular ion of m/z 421 for compound **4** (Fig.3) fragmented further along two various pathways as illustrated in (scheme 3). The molecular ion of m/z 421 underwent fragmentation via pathway A to produce a peak at m/z 224. The ion of m/z 224 was broken to give an ion of m/z 196. This fragmentation led to fragments of m/z 168, 89 and 63, respectively.

Subsequently the molecular ion of m/z 421 fragmented via the suggested pathway B to a fragmented ion of m/z 197, which further fragmented and gave a fragment of m/z 118 by losing bromine atom. The fragment of m/z 118 was broken to give fragment of m/z 102 which lost oxygen atom. It further underwent loss cyano group (CN) to give peak at m/z 76. The M+2 peak was also observed in mass spectrum for compound **4** at m/z 423 along with the molecular ion peak due to the presence of isotopes of bromine atom present in the compound. From the mass spectra of compound **4**, it was found the M+2 at m/z 423 is a base peak.

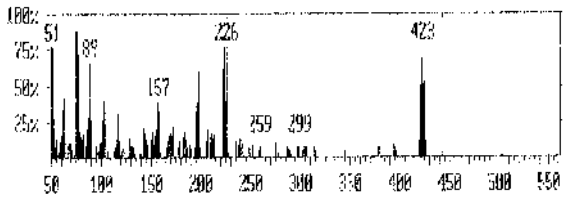
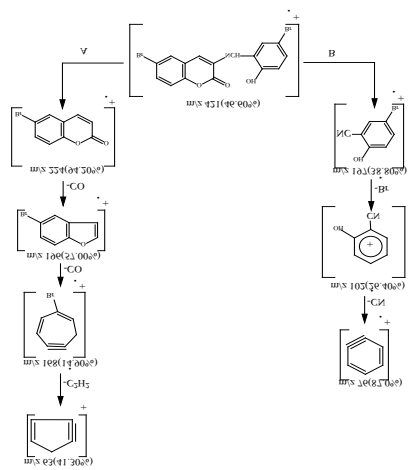


Fig. 4



Scheme 4: Main fragmentation pathways to compound 4

Compounds 5 and 6

The molecular ion of compound **5** at m/z 337 and compound 6at m/z 381 (Fig 5 and 6) had fragmented to give the fragment ion at m/z 295, corresponding to the molecular ion of compound **2b** by losing ketene and ethylene molecules. The fragment of m/z 295 was broken via pathway in the same compound **2b** (scheme **2**) .

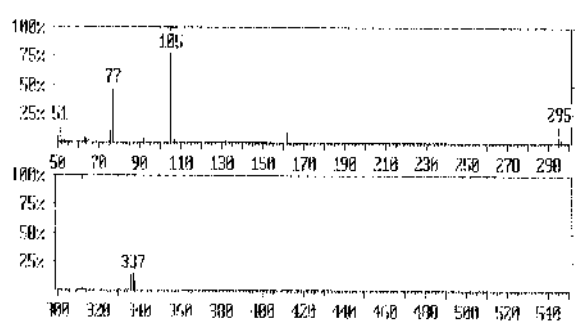


Fig. 5

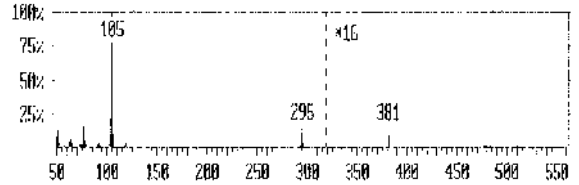


Fig. 6

Biological assay

Antimicrobial activity

All the compounds **2**, **3** and **4** were in vitro screened for their antibacterial activity against Gram-positive (bacillus subtillis, streptococcus penumonia and staphylococcus Aureas) and Gram- negative (Escherichia coli and pseudomonas sp.) bacteria. Also these compounds were tested in vitro for their antifungal activity against some fungi such as Aspergillus nigaer and penicillium Sp. by the drug diffusion method [10, 11]. The zone of inhibition was measured in mm and was compared with standard drug. DMSO was used as a blank and streptomycin, ketoconazole were used as antibacterial and antifungal standards. All the compounds were tested at 10 mg, 50 mg, and 100 mg concentration. The data are summarized in table 1, an show that all compounds display certain antimicrobial activity. The results obtained indicate that compound **3** is comparatively more active than another componds (table 1).

compound	Zone of inhibition in mm																				
	B.subtillis			s.penumonia			S.Aureas			E.coli			Pseudomonas sp.			A.Nigaer			Penicillium sp.		
	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg
2a	-	-	5	-	-	5	-	-	2	-	-	-	-	-	-	-	-	3	-	-	-
2b	-	-	9	-	1	12	-	-	13	-	-	5	-	-	3	-	-	1	-	-	-
2c	-	-	-	-	-	5	-	8	11	-	-	3	-	-	9	-	-	-	-	5	9
3	1	6	28	3	10	30	6	6	12	28	5	11	1	5	12	5	12	24	-	5	12
4	-	9	15	-	-	8	-	13	22	-	10	22	5	17	32	1	9	21	3	11	20
Streptomycin	3	7	18	2	11	17	4	16	20	8	17	22	6	12	27	-	-	-	-	-	-
ketoconazol																8	13	18	7	17	21

Table 1: Antimicrobial activity of some prepared compounds 2-4

(-)= no activity

Experimental

NMR spectra were recorded on general Electric QE300 instrument. Chemical shifts were given with respect to TMS. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a biorad FTS7 (KBr). 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. Melting points were determined on a MEL-TEMP II apparatus and uncorrected.

3, 6, 8-Trisubstituted coumarins (2a-c)

A mixture of aldehydes (namely, 5-bromo-2-hydroxybenzaldehyde and 3-methoxy-2-hydroxybenzaldehyde, (0.01 mole), N-aroylglycine (such as N-benzoylglycine and N-(P-chlorobenzoyl)glycine, 0.01 mole) and fused sodium acetate(0.03 mole) in acetic anhydride (5mL) was fused on a hot- plate for 30-45min. The reaction mixture was cooled and poured into water. The resulting solid was filtered off, washed with water, dried and recrystallized from a suitable solvent to give **2**.

3-(benzoyl)amino-6-bromocoumarin(2a),

as yellow crystals, yield 78 %, m.p 220 °C . IR (KBr): 3215 (NH), 1725 (C=O), 1605, 1585 (C=O), 1217, 1093(C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ7.12-7.81(m, 8H, Ar-H), 8.42(s, 1H, H-pyrane), 10.35(s, 1H, NH)ppm. MS:m/z(%)= 345(M^++2, 2.50), 344(M^++1, 1.7), 343(M^+,2.70), 212(0.30), 201(0.50), 200(0.5), 184(0.50), 18390.90), 182(0.60), 181(0.50), 157(0.50), 155(0.80), 107(0.60), 106(8.70), 105(100), 104(25.90), 103(2.10), 102(1.80), 92(0.30), 91(0.30), 87(0.60), 86(0.90), 78(3.30), 77(48.70), 76(10.80), 75(4.80), 64(0.500, 63(1.00), 62(1.30), 52(1.80), 51(13.30), 50(7.40). Anal. Calcd for $C_{16}H_{10}NBrO_3$: C, 55.97, H, 2.26; N, 4.08. Found: C, 55.77; H, 2.08; N, 3.98.

3-(Benzoyl) amino-8-methoxycoumarin (2b),

as yellow crystals, yield 73%, m.p 210°C. IR (KBr): 3220 (NH), 1721(C=O), 1611, 1583(C=C), 1220, 1151, 1083(C-O) cm^{-1}. ^{1}H-NMR (DNSO-d_6): δ 3.92(s, 3H, OCH_3), 7.11-7.78(m, 8H, Ar-H), 8.33(s, 1H, H-pyrane), 10.32(s, 1H, NH) ppm. MS:m/z(%)= 296(M^++1, 4.10), 295(15.50), 294(M^+-1, 0.50), 282(0.60), 281(0.60), 192(0.80), 191(1.20), 190(1.00), 163(1.40), 162(9.50), 161(1.20), 152(1.10), 149(1.50), 147(0.90), 134(1.10), 133(2.10), 132(2.80), 131(1.20), 122(1.10), 121(1.40), 107(4.10), 106(9.40), 105(100), 104(5.80), 92(3.20), 91(1.80), 90(1.40), 83(1.20), 81(2.20), 78(61.50), 77(46.50), 76(4.90), 65(5.00), 64(3.30), 63(4.40), 51(13.00), 50(4.60). Anal.Caled for $C_{17}H_{13}NO_4$:C, 69.15; H, 4.41; N, 4.74. Found: C, 69.02; H, 4.24; N, 4.56.

3-(P- Chlorobenzoyl) amino-8-methoxycoumarin (2c)

as yellow crystals, yield 74%, m.p 195 °C.IR(KBr); 3220(NH), 1723(C=O), 1698(C=O), 1605, 1583(C=C), 1215, 1121, 1095(C-O)cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ7.21-7.78(m, 7H, Ar-H), 8.39(s, 1H, H-pyrane), 3.93(s, 3H,OCH_3),10.36(s, 1H, NH) ppm. MS: M/Z(%)= 330(M^++1, 5.40), 329(M^+, 20.10), 328(M^+-1, 14.70), 312(1.20), 301(1.50), 298(1.20), 297(2.20), 279(2.00), 278(1.50), 258(1.70), 257(2.90), 226(2.50), 225(1.70), 201(1.50), 200(1.70), 199(2.20), 191(2.50), 185(2.40), 184(2.00), 172(2.00), 168(3.20), 167(4.40), 149(3.70), 148(2.50), 141(31.60), 140(14.20), 139(100), 138(20.30),, 121(2.20),, 120(4.70), 119(10.00), 118(3.20), 113(9.10), 111(25.50), 110(8.60), 105(3.90), 104(5.40), 92(7.80), 91(4.90), 90(3.20), 77(14.20), 76(9.30), 75(14.00), 65(6.50), 64(8.60), 63(8.60), 51(17.80), 50(10.80). Anal. Called for $C_{17}H_{12}NClO_4$: C, 62.01; H, 3.65; N, 4.25. Found: C, 61.98; H, 3.51; N, 4.09.

3-Amino-6-bromo coumarin (3)

Method A

A solution of **2a** (0.01 mole) in 6 N HCl (15 ml) and acetic acid (10 ml) was heated under reflux for 2-3h, then cooled poured into ice-water. The solid formed was filtered off, washed with hot water, dried and purified by recrystallization with ethanol to give 3 as pale yellow crystals, m.p. 193°C. IR (KBr): 3371, 3157(NH_2), 1728(C=O), 612, 1581(C=C), 1215, 1083(C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 5.83(S, 2H, NH2), 7.51-7.19(M, 3H, Ar-H), 8.36(S, 1H, H-pyrane). Ppm. MS: m/z(%)=241(M^++2, 17.50), 239(M^+, 24.60), 238(M^+-1, 15.80), 214(10.50&0, 212(17.50), 210(14.00), 196(12.30), 195(36.80), 194(17.50), 191(29.80), 190(19.30), 189(33.30), 188(29.80), 187(28.10), 186(19.30), 180(36.80), 179(33.30), 178(19.30), 167(15.80), 163(19.30), 162(56.10), 161(40.40), 152(22.80), 151(22.80), 150(14.00), 136(17.50), 134(38.60), 123(17.50), 121(100), 120(26.30), 111(22.80), 109(15.80), 108(19.30), 107(15.80), 106(21.10), 105(17.50), 103(22.80), 102(29.80), 93(19.30), 89(21.10), 88(26.30), 79(15.80), 78(59.60), 77(9.60), 64(50.90), 68(29.80), 67(26.30), 66(24.60), 51(19.00), 50(12.30). Anal.calcd for C9H6NBrO2: C, 45.19; H, 2.15; N, 5.86. Found: C, 45.02; H, 2.42; N, 5.68.

Method B

A mixture of 1(0.01 mole), methyl glycine ester hydrochloride (0.01 mole) and fused sodium acetate (0.03 mole) in glacial acetic acid (20 ml) was heated under reflux for 2 hr, Then cooled and poured into water. The crude product was filtered off, washed with water, dried and purified by recrystallization with ethanol to give **3**.

3-(5-Bromo-2-hydroxybenzylidene) amino-6-bromocoumarin (4)

A mixture of **3** (0.01 mole), 5-bromo-2-hydoxybenzaldehyde(0.01 mole) and fused sodium acetate(0.03 mole) in acetic acid(20 ml) was heated under reflux for 2-3 hr, then cooled and poured into water. The solid obtained was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 4 as orange crystals, yield 78%, m.p

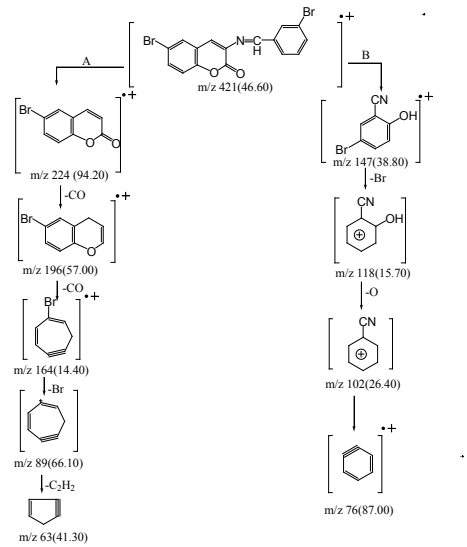
233°C.IR(KBr):3410-2850(br.OH),1727(C=O),1625(C=N), 165,1580(C=C), 1215,1125, 1087(C-O)cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 7.32-8.21(m, 7H, ArH and H-pyrane), 8.71(s, 1H, CH=N), 11.35(br-s,1H, OH)ppm. MS: m/z(%)= 425(M+4, 52.10), 423(M+2, 98.30), 421(M$^+$, 49.60),420(m$^+$-1, 32.20), 380(9.90), 379(6.60), 378(6.60), 315(4.10), 314(7.40), 306(6.60), 305(7.40), 304(8.30), 260(6.60), 259(11.60), 258(4.10), 241(18.20), 240(13.20), 234(12.40), 235(11.60), 225(38.60), 224(99.20), 223(62.00), 226(100), 212(13.20), 211(16.50), 210(14.00), 207(19.00), 206(11.60), 198(59.50), 197(38.80), 196(57.00), 195(32.20), 185(12.40), 184(18.20), 183(14.00), 172(20.70), 171(14.90), 170(16.50), 169(15.70), 168(14.90), 167(11.60), 158(31.40), 157(38.80), 156(24.00), 155(19.00), 154(15.70), 152(17.40), 151(13.30), 145(12.40), 144(16.50), 143(20.70) 129(14.00), 119(10.70), 118(15.70), 117(29.80), 116(12.90), 105(18.20), 104(3.90), 103(39.70), 102(26.40), 90(26.40), 89(66.10), 88(45.50), 87(28.10), 81(12.40), , 80(14.00), 79(18.20), 78(14.00), 77(71.10), 76(87.60), 75(88.40), 74(37.20), 64(13.20), 63(41.30), 62(33.10), 51(96.60), 50(40.10). Anal. Calcd for $C_{16}H_9NBr_2O_3$: 45.60; H, 2.14; N, 3.32. Found: C, 45.43; H, 2.02; N, 3.11

3-(N-Acetyl-N-benzoyl)amino-8-methoxycoumarin (5)

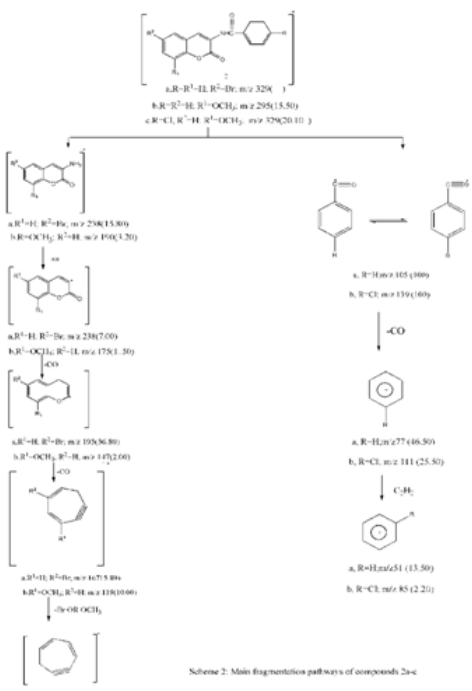
A solution of **2b** (0.01 mole) in acetic anhydride (25 ml) d was heated under reflux for 2h, then cooled and poured into water. The resulting product was filtered off, washed with water, dried and purified by recrystallization with ethanol to give 5 as yellow crystals, yield 67%, m.p. 175 °C. IR (KBr): 1725, 1697(C=O), 1610, 1580(C=C), 1221, 1078(C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 2.31(s, 3H, CH$_3$), 3.43(s, 3H, OCH$_3$), 7.12-7.89(m.8H, Ar-H), 8.31(s, 1H, H-pyrane)ppm. MS: m/z (%) = 338(M$^+$+1, 2.10), 337(M$^+$, 4.50), 296(2.60), 295(14.90), 294(1.10), 163(1.00), 162(8.60), 161(1.00), 133(1.50), 132(1.40), 119(2.00), 118(0.70), 107(3.50), 106(10.20), 105(100), 103(1.30), 92(3.20), 91(1.00), 90(0.80), 78(4.00), 77(46.00), 76(9.70), 75(1.60), 65(2.90), 64(3.40), 63(4.20), 51(13.10), 50(5.20),. Anal. Calcd for $C_{17}H_{15}NO_5$: C, 60.53; H, 4.45; N, 4.15. Found: C, 6.35; H, 4.28; N, 4.03.

3-(N-Ethoxy carbonyl methyl-N-benzoyl) amino-8- methoxycoumarin (6)

A mixture of **2b** (0.01 mole), ethyl chloroacetate (0.01 mole) and fused sodium acetate (0.03 mole) in acetic acid (30 ml) was heated under reflux for 4h. The reaction was cooled and poured into water. The crude product obtained was filtered, washed with water, dried and purified by recrystallization with ethanol to give **6** as pale yellow crystals, yield 71%, m.p. 165 °C.IR(KBr): 1755, 1722, 1697(C=O), 1611, 1583(C=C), 1225, 1125, 1075(C-O) cm^{-1}. ^{1}H-NMR (DMSO-d$_6$): δ 1.3(t, 3H, CH3), 3.94(s, 3H, OCH3), 4.51(q, 2H, OCH2), 7.11-7.81(m, 8H, Ar-H), 8.33(s, 1H, H-pyrane) ppm. MS: m/z (%) = 382(19.20), 381(22.300, 296(3.30), 295(15.00), 294(9.10), 251(1.30), 191(1.30), 121(0.80), 119(2.40), 106(10.20), 105(100), 104(21.20), 103(3.10), 94(1.60), 93(1.10), 92(3.50), 91(2.00), 78(3.80), 77(57.60), 76(15.70), 63(4.20), 62(3.30), 51(2.60), 50(6.80), Anal.Calcd for C21H19NO6 : C, 66.14; H, 4.99; N, 3.67. Found: C, 66.02; H, 4.78; N, 3.42.



Scheme 3 : Main fragmentation pathway of compound 4



Scheme 2: Main fragmentation pathways of compounds 2a-c

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