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Synthesis of novel amino and acetyl amino-4-methylcoumarins and evaluation of their antioxidant activity

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Abstract

The six novel 4-methylcoumarins bearing different functionalities such as amino, hydroxy, *N*-acetyl, acetoxy and nitro have been synthesized and confirmed on the basis of their spectral data (^{1}H-, ^{13}C-NMR, UV, IR and EI mass). They were examined for the first time for their effect on NADPH dependent liver microsomal lipid peroxidation in vitro, and the results were compared with other model 4-methylcoumarin derivatives to establish the structure–activity relationship. Our studies demonstrated that amino group is an effective substitute for the hydroxyl group for antioxidant property and produced a dramatic inhibition of lipid peroxidation. Ortho dihydroxy and ortho hydroxy-amino coumarins were found to possess highest antioxidant and radical scavenging activities.

Keywords: Acetoxy-4-methyl coumarin; Amino-4-methyl coumarin; Antioxidant; Coumarins lipid peroxidation; Radical scavenging; Reactive oxygen species; Free radicals

1. Introduction

Coumarins and their derivatives have been found to exhibit different biological and pharmacological activities [1]. The 4-methylcoumarins have been found to possess anti-inflammatory [2], cholesretic [3], analgesic [4], antispermatogenic [5] and diuretic [6] properties. Apart from the medicinal applications coumarins are also used as sweetener, fixative of perfumes [7], enhancer of natural oils such as lavender, a food additive in combination with vanillin, a flavour/odour stabilizer in tobacco [7], an odour masker in paints and rubber. Owning to the widespread applications, synthetic and biological activity evaluation of coumarins and their derivatives has been a subject of intense investigations.

The importance of free radicals, especially reactive oxygen species (ROS) in the pathogenicity of various diseases [8,9], including hepatic and vascular diseases [10] has of late received greater attention. Antioxidants are now forged as the drug candidates to combat these diseases. Minor dietary constituents have been seriously considered to counter the ill effects of the oxygen radicals. The 4-methyl coumarins are known to be less toxic compared to coumarins. In this report, we have examined 4-methyl coumarin possessing dihydroxy, diacetoxy and hydroxy-amino groups in the benzenoid ring at positions ortho to each other, they have shown very good antioxidant and radical scavenging properties, also comparatively better than those of α-tocopherol.

2. Materials and methods

2.1. Chemicals

Nicotinamide dinucleotide phosphate (NADPH), adenosine diphosphate (ADP), acetic anhydride, pyridine and trichlo-

Abbreviations: Ac_2O, acetic anhydride; Py, pyridine.

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roacetic acid (TCA) were obtained from Sisco Research Laboratory (Mumbai, India), tris, $FeCl_3$, thiobarbituric acid (TBA), dimethyl sulfoxide (DMSO), ammonium hydroxide and sodium hydrosulfite of high purity were purchased from local suppliers. Diphenyl picryl hydrazyl (DPPH) was procured from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Animals

Male albino rats of wistar strain weighing around 180–200 g, fed on rat chow supplied by Hindustan Lever Ltd., Mumbai, India were used.

2.3. Chemistry procedures

2.3.1. 4-Methyl coumarin derivatives

We have prepared several 4-methyl coumarin derivatives and given chemical procedure for all the 4-methyl derivatives (given under Sections 2.3.2–2.3.8). There is no as such novel 4-methyl coumarin derivatives for chemistry procedure except given below.

2.3.2. 7,8-Dihydroxy-4-methylcoumarin DHMC, 2a

The 7,8-dihydroxy-4-methyl coumarin was prepared by well-known Pechmann condensation of pyragallol with ethyl acetoacetate [11], its diacetoxy derivative 7,8-diacetoxy-4-methyl coumarin, DAMC 3a was prepared by the acetylation of DHMC with acetic anhydride and pyridine.

2.3.3. 8-N-acetyl-7-acetoxy-4-methylcoumarin 8

To an ice-cold solution of compound 4 (4.85 g, 0.025 mol) in 60 ml of concentrated ammonium hydroxide, a cold solution sodium hydrosulfite (75 g) in 300 ml of water was added rapidly and reaction mixture was stirred for 90 min. A yellow precipitate of m.p. 254–256 °C was formed (70–75% yield) which was crystallized by ethanol to obtain pure crystalline product 6. The acetoxy derivative of amino-hydroxy coumarin 6 (2 g, 10.47 mmol) was synthesized by the acetylation with acetic anhydride (2.2 ml, 20.14 mmol) and pyridine (1.16 ml, 15.36 mmol) and stirred the reaction mixture for 5–6 h and progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was poured over crushed ice. The precipitate was filtered, dried over P_2O_5 in a vacuum desiccator and the crude product was purified by crystallization and afforded the compound 8 in crystalline form.

2.3.4. 5-N-acetyl-6-acetoxy-4-methylcoumarin 9

Obtained as white crystals from compound 7 (2 g, 10.47 mmol) according to a procedure already described in compound 8.

2.3.5. 5,7-Dinitro-6-acetoxy-4-methylcoumarin 11

To stirred solution of compound 2c (10 g, 0.057 mol) in 80 ml of concentrated sulfuric acid, a solution of nitration mixture (70 % HNO_3, 5,08 g, 0.057 mol, in 12 ml of concentrated sulfuric acid) was added and reaction mixture was stirred for the 1 h at 2–4 °C. The cold reaction mixture was poured over crushed ice (1 kg), and then a yellow precipitate was filtered, washed with cold water (40 ml) dried over P_2O_5 in vacuum desiccator and column chromatographed over silica gel using ethyl acetate-petroleum ether as eluent to obtain compound 10 [12] in almost quantitative yields. The acetate (11) of respective hydroxy derivative 10 (5,0 g, 0.019 mol) was prepared using Ac_2O/Py as described in compound 8.

2.3.6. 5,7-Diamino-6-hydroxy-4-methylcoumarin 12

Greenish powder from compound 10 (3.0 g, 11.27 mmol), prepared as described in compound 8.

2.3.7. 5,7-N-diacetyl-6-acetoxy-4-methylcoumarin 13

Prepared as white colored powder from compound 12 (2.0 g, 9.7 mmol) by procedure described in compound 8.

2.3.8. 7,8-Diacetoxy-6-nitro-4-methylcoumarin 15

White colored powder from compound 14 (2.0 g, 8.43 mmol), prepared as described in compound 8.

2.4. Biological tests

2.4.1. Preparation of rat liver microsomes

Rat liver microsomes used for lipid peroxidation studies were prepared adopting the method of Ernster and Nordenbrand [13]. Freshly excised rat liver was suspended in 0.25 M sucrose solution and homogenized to obtain 30% homogenate which was centrifuged at 10,000 g for 30 min in a Sorvall refrigerated centrifuge. The supernatant was spun at 100,000 g for 1 h in Beckman ultracentrifuge (model L) and the surface of pelleted microsomes was rinsed with 0.15 M KCl and resuspended in 0.15 M KCl. Protein was assayed by the method of Lowry et al. [14].

2.4.2. Assay for initiation of lipid peroxidation

Detailed assay procedure has been given in our earlier communication [15]. In short, rat liver microsomes (1 mg protein) were preincubated with Tris–HCl (0.025 M, pH 7.5) and test compound (100 μM, in DMSO) was added and incubated at 37 °C for 10 min followed by the addition of ADP (3 mM) and $FeCl_3$ (0.15 mM). The initiation of enzymatic lipid peroxidation was started by the addition of NADPH (0.5 mM) and incubation of the reaction mixture continued for 10 min. The products of lipid peroxidation were quantified by estimation of thiobarbituric acid reactive substances (TBARS) thus formed as described earlier.

2.4.3. Assay for DPPH radical scavenging

A solution of test compounds in methanol (4.0 ml) at various concentrations ranging from 1 to 400 μM depending on the potency of the inhibitor was added to 1.0 ml of DPPH solution in methanol (0.15 mM). The contents were vigorously mixed, allowed to stand at 20 °C for 30 min and the absorption was read at 517 nm.

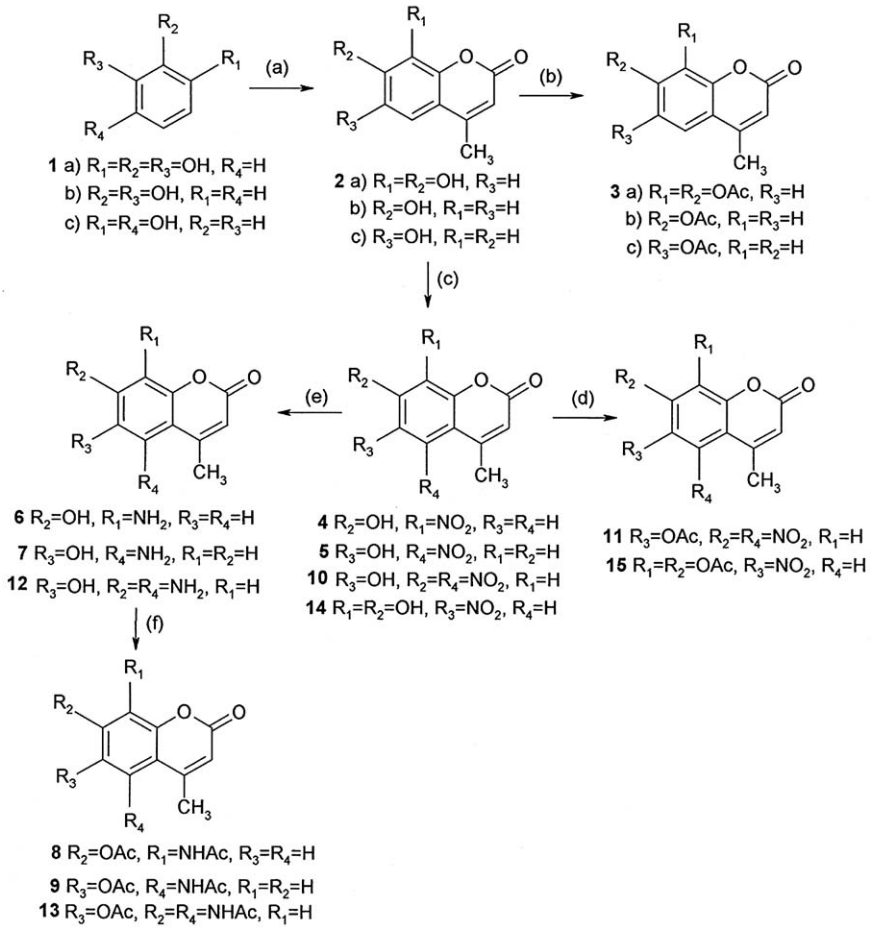
2.4.4. Calculation of IC_{50}

The different concentrations of inhibitor ranging from 0.01 to 100 μM were incubated as described above to calculate the inhibitor concentration for 50% inhibition (IC_{50}).

3. Results and discussion

The coumarins (2–15) were synthesized by the well-known Pechmann condensation [11] in quantitative yield. The compounds 8-*N*-acetyl-7-acetoxy-4-methylcoumarin (8), 5-*N*-acetyl-6-acetoxy-4-methylcoumarin (9), 5,7 -dinitro-6-acetoxy-4-methylcoumarin (11), 5,7-diamino-6-hydroxy-4-methylcoumarin (12), 5,7-*N*-diacetyl-6-acetoxy-4-methylcoumarin (13), and 7,8-diacetoxy-6-nitro-4-methylcoumarin (15) have been synthesized and reported for the first time (Scheme 1). Structural confirmation was done using ^{1}H NMR, ^{13}C NMR, UV, IR and EI Mass spectrum. The spectral details for these compounds are given under Sections 2.3.1–2.3.8.

The IR spectrum of compounds 8 and 9 showed three characteristic absorptions at 1769, 1735, 1658 and 1771, 1734, 1649 cm^{-1} for carbonyl groups. The ^{1}H NMR spectrum showed characteristic peak of 4-methyl coumarin at δ 6.30 and 6.01 for C-3H in both compounds 8 and 9. In both cases, two doublets appeared at δ 7.21 and 7.42 for compound 8 (assigned for C-5H and C-6H) and at δ 7.01 and 7.12 for compound 9 (assigned for C-7H and C-8H), respectively. The ^{13}C NMR spectrum showed C-2 at δ 159.08 and 157.10 for compounds 8 and 9, respectively. Finally, the structure was supported by their EIMS data, the [M + 1] peak appeared at *m*/*z* 276 for both the compounds. The IR spectrum of compound 11 showed two carbonyl absorptions at 1803 and 1743 cm^{-1}. The ^{1}H NMR spectrum characteristic singlet of 4-methyl coumarins appeared at δ 6.56 for C-3H proton. Finally, the structure was supported by its ^{13}C NMR spectrum, C-2 appeared at δ 156.56 and other carbonyl carbon appeared at δ 167.02. The compounds 12 showed three characteristic absorptions at 3433, 3353 and 1737 cm^{-1} for hydroxyl, amino, and carbonyl groups in its IR spectrum. The ^{1}H NMR spectrum showed characteristic singlet of 4-methyl coumarins at δ 6.41 for C-3H proton. In its ^{13}C NMR spectrum, C-2 appeared at δ 158.06 and finally the structure is confirmed by its EIMS data, it showed $[M]^+$ peak at *m*/*z* 206. The compound 13 had three characteristic absorptions at 1740, 1701 and 1665 cm^{-1} for *N*-acetyl, acetoxy and carbonyl groups in its IR spectrum. The ^{1}H NMR spectrum showed characteristic singlet of 4-methyl coumarins at δ 6.30 for C-3H proton. In its ^{13}C NMR



Scheme 1. (a) $CH_3COCH_2COOC_2H_5$/cold H_2SO_4; (b) AC_2O/Py; (c) HNO_3/H_2SO_4; (d) AC_2O/Py; (e) solid hydrodisulfite/concentrated NH_4OH; (f) AC_2O/Py. All the compounds were characterized by mp, spectroscopic data (UV, IR, NMR, MS).

spectrum C-2 appeared at δ 157.05 and three other carbonyl appeared at δ 166.21, 167.12 and 167.25 for carbonyl carbons. Finally, the MS peak at *m/z* 232 supported by its structure. The IR spectrum of compound 15 showed two characteristic absorptions at 1788 and 1740 cm^{-1} for two carbonyl groups. The ^1H NMR spectrum showed characteristic singlet of 4-methyl coumarins appeared at δ 6.10 for C-3H proton and finally the structure was confirmed by its ^{13}C NMR spectrum, C-2 appeared at δ 156.90 and other two carbonyl carbons appeared at δ 167.10, 167.61.

3.1. Chemistry

The synthesis of the novel compounds is outlined in Scheme 1. The preparation of derivatives of nitro and amino coumarins is based on the methodology used by Kaufman et al. [12] with some modifications. The sequence of reactions started from compound 2. Basic nitration of 2a–c afforded the nitro derivatives 4, 5, 10 and 14, compounds 10 and 14 were treated with acetic anhydride/pyridine (Ac_2O/Py) to obtain the compounds 11 and 15, respectively, in quantitative yield. On the other hand, according to Kaufman et al. amino derivatives 6, 7 and 12 were prepared by the reduction of respective nitro derivatives 4, 5 and 10 with the concentrated ammonium hydroxide and sodium hydrosulfite, after stirring the reaction mixture for 90 min. The compounds 8, 9 and 13 were obtained by treating 6, 7 and 12 with Ac_2O/Py mixture at 25 °C. The spectral data of all the novel compounds are given below.

3.1.1. 4-Methyl coumarin derivatives

All synthesized compounds are 4-methyl coumarin derivatives and there is no as such novel 4-methyl coumarin derivatives for spectral data except given below.

3.1.2. 7,8-Dihydroxy-4-methylcoumarin DHMC, 2a

The structure of DHMC was confirmed as per reported spectral data [11] and the structure of DAMC was confirmed by spectral data reported earlier [16].

3.1.3. 8-N-acetyl-7-acetoxy-4-methylcoumarin 8

Yield: 2.01 g (70% yield). m.p.: 185–187 °C; UV: λ_{max} 276, 215.; IR (Nujol): V_{max} 3230, 2928, 1906, 1769, 1735, 1658, 1606, 1566, 1529, 1462, 1373, 1354, 1233, 1115, 1060, 947, 858, 720, 640, 523 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.21 (3H, s, $OCOCH_3$), 2.30 (3H, s, $NHCOCH_3$), 2.42 (3H, s, C-4 CH_3), 6.30 (1H, s, C-3H),7.21 (1H, d, C-5H) 7.42 (1H, d, C-6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.80, 22.23 and 22.80 (C-7OCO**C**H_3, C-8NHCO**C**H_3, and C-4CH_3,), 116.43, 117.20 and 118.78 (C-5, C-6, C-3), 126.10 and 127.95 (C-10, C-9), 144.27 (C-4), 151.67 and 152.66 (C-8, C-7), 159.08 (C-2, C=O), 168.55 and 169.70 (C-8 and C-7, 2XC=O); EIMS: *m/z* (rel. int.) 276 [M + 1] (10%), 233 (45%), 215 (100%), 162 (62%), 134 (32%), 106 (22%), 89 (13%), 77 (30%), 58 (32%), 43 (100%).

3.1.4. 5-N-acetyl-6-acetoxy-4-methylcoumarin 9

Yield: 2.25 g (78%). m.p.: 170–172 °C; UV (MeOH): λ_{max} 285, 253 nm; IR (KBr): V_{max} 3230, 3087, 3006, 2367, 1771, 1734, 1649, 1566, 1527, 1443, 1372, 1273, 1115, 1059, 1017, 974, 947, 721, 523, and 431 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$): δ 2.11 (3H, s, CH_3-CO), 2.22 (3H, s, 3H, s, NH-$COCH_3$), 2.40 (3H, s, C-4 CH_3), 6.01 (1H, s, C-3H), 7.01 (1H, d, J = 8.4 Hz, C-7H) 7.12 (1H, d, J = 8.4 Hz, C-8H); ^{13}C NMR (75,5 MHz, $CDCl_3$): δ 18.76, 20.18 and 20.75 (C-6$OCOCH_3$, C-5$NHCOCH_3$ and C-4CH_3), 114.38, 115.16 and 116.73 (C-7, C-8, C-3), 124.06 and 125.90 (C-10, C-9), 142.22 (C-4), 149.62 and 150.62 (C-5, C-6), 157.10 (C-2, C=O), 166.50 and 167.65 (C-5, C-6, 2xC=O); EIMS, *m/z* (rel. int.) 276 [M + 1] (8%), 233 (43%), 215 (100%), 162 (62%) 134 (30%), 106 (18%), 89 (10%), 79 (26%), 53 (20%), 43 (100%).

3.1.5. 5,7-Dinitro-6-acetoxy-4-methylcoumarin 11

Yield 5.04 g (87%). m.p.: 205 °C; UV (MeOH): λ_{max} 268, 208; IR (Nujol): V_{max} 2922, 1803, 1765, 1743, 1532, 1463, 1412, 1375, 1340, 1311, 1222, 1190, 1053, 975, 892; ^1H NMR (300 MHz, $CDCl_3$): δ 2.38 (6H, s, 3H each, CH_3 and $OCOCH_3$), 6.56 (1H, s, C-3H) and 8.24 (1H, s, C-8H); ^{13}C NMR (75,5 MHz, $CDCl_3$): δ 18.27 and 20.27 (C-4CH_3 and C-6$COCH_3$), 116.08 and 116.94 (C-3, C-8), 122.20 and 123.00 (C-9, C-10), 133.31 (C-4), 142.08, 146.82 and 150.69 (C-5, C-7, C-6), 156.56 (C-2, C=O), 167.02 (C-6, C=O).

3.1.6. 5,7-Diamino-6-hydroxy-4-methylcoumarin 12

Yield 1.62 g (70%). m.p.: >260 °C; UV: MeOH λ_{max} 311, 258, 240; IR (Nujol): V_{max} 3433, 3353, 2925, 1737, 1633, 1552, 1461, 1377, 1292, 1243, 1142, 1084, 922, 853, 757, 691, 54 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.58 (3H, s, C-4, CH_3), 6.41 (1H, s, C-3H), 9.50 (1H, s, C-8H); ^{13}C NMR (75,5 MHz, DMSO-d_6): δ 18.25, (C-4CH_3), 115.35 and 117.59 (C-3) and (C-8), 121.22 and 122.70 (C-9 and C-10), 133.31(C-4), 141.68, 145.42 and 147.42 (C-5, C-7 and C-6), 158. 06 (C-2, C=O); EIMS, *m/z* (rel. int.): 206 [M^+] (4%), 191 (100%), 162 (59%), 148 (14%), 134 (18%), 128 (8%), 58 (100%) 53 (18%).

3.1.7. 5,7-N-diacetyl-6-acetoxy-4-methylcoumarin 13

Yield 1.29 g (40%). m.p.: > 260 °C; UV (MeOH): λ_{max} 330, 298, 214 nm; IR (KBr): V_{max} 3342, 3243, 2931, 1740, 1701, 1665, 1615, 1574, 1462, 1371, 1251, 1197, 1099, 1072, 942, 865, 693, 596, 456 cm^{-1}. ^1H NMR (60 MHz, DMSO-d_6): δ 2.29 (3H, s, $OCOCH_3$), 2.40 (6H, s, $NHCOCH_3$), 2.49 (3H, s, C-4 CH_3), 6.30 (1H, s, C-3H), and 8.23 (1H, s, C-8H); ^{13}C NMR (75,5 MHz, DMSO-d_6): δ 18.79, 19.72, 20.43 and 22.01 (C-4CH_3, C-5$NHCOCH_3$, C-7$NHCOCH_3$ and C-6$OCOCH_3$), 111.54 and 113.20 (C-8, C-3), 125.56 and 126.15 (C-10, C-9), 133.04 (C-4), 149.50, 149.95 and 150.25 (C-5, C-7, C-6), 157.05 (C-2, C=O), 166.21, 167.12 and 167.25 (C-5, C-7, C-6, 3xC=O). EIMS *m/z* (rel. int.) 332 [M^+] (100%), 299 (71%), 268 (4%), 255 (8%), 199 (6%), 143 (19%), 129 (6%), 87 (76%), 74 (100%), 69 (21%), 57 (28%), 43 (45%).

3.1.8. 7,8-Diacetoxy-6-nitro-4-methylcoumarin 15

Yield 2.16 g (80%). m.p.: 149–151 °C; UV (MeOH): λ_{max} 279, 266; IR (Nujol): V_{max} 2923, 1788, 1740, 1619, 1567, 1455, 1374, 1288, 1245, 1040, 1010, 922, 854, 795 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$): δ 2.22 (6H, s, 2XOCH_3) 2.31 (1H, s, CH_3), 6.10 (1H, s, C-3H) and 7.11 (1H, s, C-5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.20, 19.52 and 19.93 (C-4**C**H_3, C-7 and C-8 2x$COCH_3$), 111.53 and 114.63 (C-3 and C-5), 115.07 and 119.26 (C-9 and C-10), 133.51 (C-4), 144.92, 148.86 and 148.98 (C-6, C-7 and C-8), 156.90 (C-2, C=O), 167.10 and 167.61 (C-7 and C-8, 2xC=O).

3.2. Biological activity

3.2.1. Assay for initiation of lipid peroxidation and DPPH

Assay procedures for initiation of lipid peroxidation and DPPH were carried out by using our reported procedure [15]. The results are shown in Tables 1,2,3, respectively.

3.2.2. IC_{50} value determination

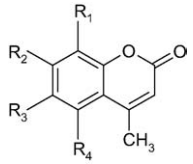
The different amount of inhibitor ranging from 0.1 to 100 μM was incubated as described in our earlier publication [15] to calculate the inhibitor concentration for 50% inhibition (IC_{50}). The results are shown in Table 4.

3.2.3. Antioxidant activity studies

Fourteen compounds along with two model compounds (DHMC & DAMC) were screened for their effect on rat liver microsomal lipid peroxidation. Svingen et al. [17] had demonstrated that NADPH-dependent lipid peroxidation proceeds through the formation of lipid hydro peroxides, called initiation step. We have investigated the effect of above-mentioned derivatives of 4-methyl coumarins on the initiation of membrane lipid peroxidation. The results given in Tables 1 and 3 illustrated that the ortho dihydroxy, ortho diacetoxy and ortho amino-hydroxy derivatives of 4-methyl coumarin showed very good antioxidant property. But when nitro group is introduced at the adjacent position of hydroxyl group, the hydroxy-nitro compounds (4, 5 and 10) showed much less inhibition but acetoxy-nitro derivative (11) of 4-methyl coumarin had no ability to inhibit the initiation of lipid peroxidation as compared to above-mentioned compounds. The replacement of nitro group by amino group in the compounds 6, 7 and 12 increased the activity tremendously (almost 48%). The ortho amino-hydroxy derivatives (compounds 6 and 7) caused inhibition of lipid peroxidation similar to hydroxy coumarin (2) (87%). A comparison was made with the well-known antioxidant α-tocopherol and it was observed that ortho amino-hydroxy coumarins were much better antioxidant than, vitamin-E as the former was found to have much lower IC_{50} value than the later. Normally the antioxidant property of a compound is attributed to its (a) oxygen radical scavenging ability, (b) the ability to inhibit cellular microsomal P-450 linked Mixed Function Oxidases (MFOS) ability to suppress the formation of reactive oxygen species (ROS) [18]. The mechanism pathway for the prevention of ROS formation is given in our earlier publication (Fig. 1) [19].

Acetoxy-nitro and hydroxy-nitro derivatives of 4-methyl coumarins showed no radical scavenging activity as compared to above-mentioned coumarins (Table 2). But ortho amino-hydroxy coumarins (6, 7, 12) exhibited very good antioxidant and radical scavenging property possibly by forming a stable mixed ligand complex with ADP and Fe^{2+} thereby preventing the production of ADP-perferryl radical responsible for ROS formation as postulated by us [18]. When ortho

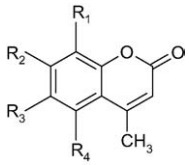
Table 1
Effect of coumarins on NADPH-dependent lipid peroxidation in rat liver microsomes



Compound number	R_1	R_2	R_3	R_4	Percent of control
(2a)	OH	OH	H	H	13
(3a)	$OCOCH_3$	$OCOCH_3$	H	H	15
(4)	NO_2	OH	H	H	60
(5)	H	H	OH	NO_2	62
(6)	NH_2	OH	H	H	12
(7)	H	H	OH	NH_2	13
(8)	$NHCOCH_3$	$OCOCH_3$	H	H	20
(9)	H	H	$OCOCH_3$	$NHCOCH_3$	20
(10)	H	NO_2	OH	NO_2	68
(11)	H	NO_2	$OCOCH_3$	NO_2	95
(12)	H	NH_2	OH	NH_2	16
(13)	H	$NHCOCH_3$	$OCOCH_3$	$NHCOCH_3$	19
(14)	OH	OH	NO_2	H	14
(15)	$OCOCH_3$	$OCOCH_3$	NO_2	H	22

The inhibitor concentration was 100 μM. The values represent mean of three separate experiments with variation of <5%.

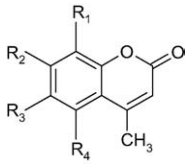
Table 2
Radical scavenging potential of some 4-methyl coumarins



Compound number	R_1	R_2	R_3	R_4	Radical scavenging (percent of control)
(2a)	OH	OH	H	H	04
(3a)	$OCOCH_3$	$OCOCH_3$	H	H	13
(4)	NO_2	OH	H	H	92
(5)	H	H	OH	NO_2	92
(6)	NH_2	OH	H	H	04
(7)	H	H	OH	NH_2	05
(8)	$NHCOCH_3$	$OCOCH_3$	H	H	54
(9)	H	H	$OCOCH_3$	$NHCOCH_3$	56
(10)	H	NO_2	OH	NO_2	95
(11)	H	NO_2	$OCOCH_3$	NO_2	94
(12)	H	NH_2	OH	NH_2	16
(13)	H	$NHCOCH_3$	$OCOCH_3$	$NHCOCH_3$	46
(14)	OH	OH	NO_2	H	10
(15)	$OCOCH_3$	$OCOCH_3$	NO_2	H	30

The inhibitor concentration was 100 μM. The values represent mean of three separate experiments with variation of <5%.

Table 3
Comparative inhibitory action of 4-methyl coumarins

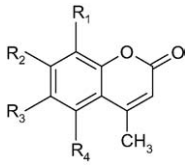


Compound number	R_1	R_2	R_3	R_4	TBARS formed (μ mol/mg protien)	Reference
Control					6.520	
(2a)	OH	OH	H	H	0.614	[15]
(3a)	$OCOCH_3$	$OCOCH_3$	H	H	0.672	[15]
(6)	NH_2	OH	H	H	0.537	
(7)	H	H	OH	NH_2	0.542	
(8)	$NHCOCH_3$	$OCOCH_3$	H	H	1.340	
(9)	H	H	$OCOCH_3$	$NHCOCH_3$	1.420	
(12)	H	NH_2	OH	NH_2	0.524	
(13)	H	$NHCOCH_3$	$OCOCH_3$	$NHCOCH_3$	1.390	
	α-Tocopherol				1.50	[15]

The lipid peroxidation procedure is given in Section 2. The inhibitors concentration was 100 μM. The values represent mean of three separate experiments with variation of <5%.

N-acetyl-acetoxy derivatives of 4-methyl coumarins (8, 9, 13) were compared with ortho di-acetoxy derivatives (3, 15), we have found comparable results for the inhibition of lipid peroxidation but in the case of radical scavenging property of *N*-acetyl-acetoxy derivatives are not comparable with DAMC (Table 2). The IC_{50} values for inhibition of lipid hydroperoxide formation (Table 4) have been evaluated only for those compounds, which have shown very high degree of inhibition of lipid peroxidation at the initiation stage. The IC_{50} values for the compounds 6, 7 & 12 were 6.02, 8.31 and 7.12, respectively (Table 4) that is comparable with DHMC (7.90) for the inhibition of the initiation of lipid peroxidation. We had shown earlier [20,21] that the ortho dihydroxy system was able to form a resonance stable radical. Similarly the ortho amino-hydroxy system could possibly form a resonance stable radical as shown in Fig. 1 responsible for the radical scavenging property of ortho amino- hydroxy-4-methyl coumarin. We had demonstrated earlier [22] the conversion of DAMC to DHMC by a specific deacetylase present in microsomes. The acetylation of the O-glycoside of *N*-hydroxy acetanilide was shown to be catalyzed by specific deacetylase localized in rat liver microsomes [23]. The deacetylation of *N*-acetyl 4-methyl

Table 4
Comparison of inhibitory potential of hydroxy and aminocoumarins



Compound number	R_1	R_2	R_3	R_4	IC_{50} (μmol)	Reference
(2a)	OH	OH	H	H	7.90	[15]
(3a)	$OCOCH_3$	$OCOCH_3$	H	H	0.25	[15]
(6)	NH_2	OH	H	H	6.02	
(7)	H	H	OH	NH_2	8.31	
(8)	$NHCOCH_3$	$OCOCH_3$	H	H	22.8	
(9)	H	H	$OCOCH_3$	$NHCOCH_3$	24.7	
(12)	H	NH_2	OH	NH_2	7.12	
(13)	H	$NHCOCH_3$	$OCOCH_3$	$NHCOCH_3$	20.01	
	α-Tocopherol				31.62	[15]

The initiation of lipid peroxidation procedure is given in Section 2. The effect of inhibitors concentration ranging from 0.01 to 100 μM on initial rate of lipid peroxidation was determined to calculate the inhibitor concentration for 50% inhibition (IC_{50}). The values represent mean of three separate experiments with variation of <5%.

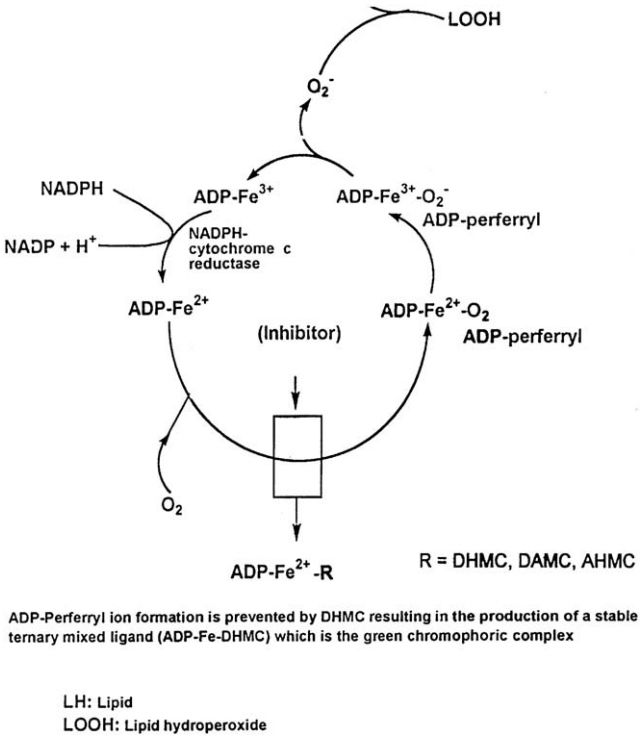


Fig. 1. Inhibition of NADPH-dependent microsomal lipid peroxidation by DHMC.

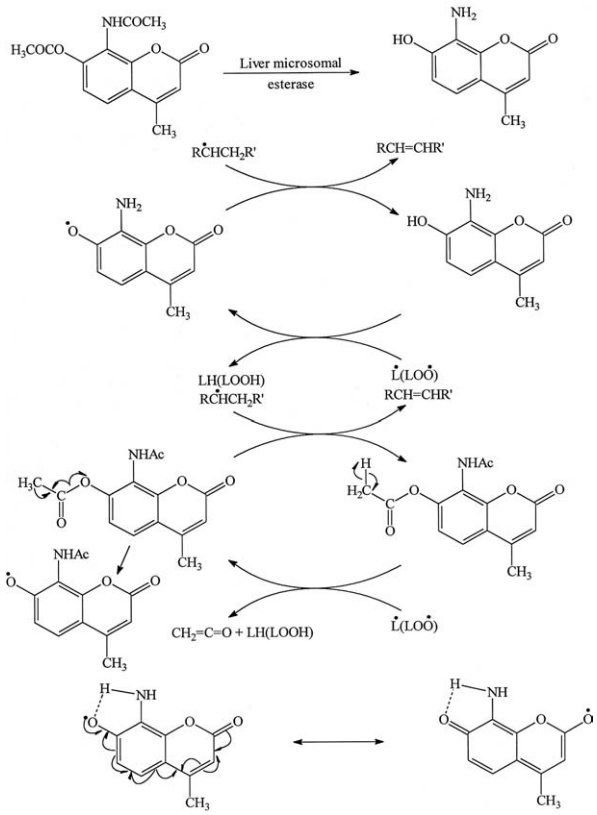


Fig. 2. Suggested reaction of *N*-acetyl and amino coumarin showing radical structures.

coumarin can be possibly catalyzed by such deacetylase mentioned above, forming DHMC, which enter the radical scavenging pathway (Fig. 2) as described in our previous publication [12].

4. Conclusions

Six novel compounds have been synthesized and characterized with the help of spectroscopic techniques and were screened for their ability to inhibit NADPH dependent lipid peroxidation of rat liver microsomes. Ortho hydroxy-amino coumarins 6, 7 and 12 were identified as potent inhibitors of lipid peroxidation, better than those of α-tocopherol. It is conceivable from these studies that the amino group can substitute for the hydroxyl group of coumarin to be effective as the inhibitor of membrane lipid peroxidation.

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