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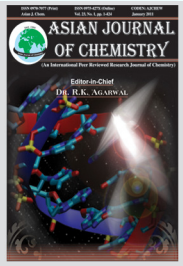

Synthesis and Spectrophotometric Determination of 7-Hydroxy-4-methyl Coumarin Containing Schiff Base Derivatives with Potential Antimicrobial and Antioxidant Activities

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2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetohydrazide (**3**), aryl/hetero aromatic aldehydes were condensed under reflux temperature for the synthesis of new Schiff base 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(substitutedmethylene)acetohydrazides (**4a-l**) and characterized through IR, ^{1}H NMR and Mass spectral data. The synthesized compounds have been screened for their antimicrobial activity in MIC levels against *E. coli*, *S. aureus* and *P. aeruginase*. The antioxidant activity of Schiff base containing coumarin moiety and its derivatives were investigated using the DPPH radical scavenging method. All these derivatives were subjected to UV-visible analysis and the molar absorptivity (ε) was determined using maximum absorbance at different wavelengths for all the compounds.

Key Words: 7-Hydroxy-4-methylcoumarin, Schiff base, Antimicrobial activity, Antioxidant activity, Spectrophotometric determination.

INTRODUCTION

Coumarin derivatives have been of great interest because of their role in natural and synthetic organic chemistry. They have varied bioactivities, such as, inhibition of platelet aggregation[1], antibacterial[2], anticancer[3], steroid 5α-reductase inhibition[4], HIV-1 protease inhibition[5], molluscicides[6], anthelmintic, hypnotic, insecticidal[7] while some derivatives serve as anticoagulants[8]. Coumarins have also been used as food additives and in cosmetics[9], as optical brightening agents[10] as well as dispersed fluorescent and laser dyes[11]. Their properties turn coumarins very interesting targets to organic chemists and several strategies for their synthesis have already been established.

Azomethine group (>C=N-) containing compounds typically known as Schiff bases have been synthesized by the condensation of primary amines with active carbonyls. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial[12-17], antifungal[14-17] and antitumor activity[18,19]. They have been studied extensively as a class of ligands[20-22] and are known to coordinate with metal ions through the azomethine nitrogen atom.

Schiff base complexes play a vital role in designing metal complexes related to synthetic and natural oxygen carriers[23]. After complexation with metals, these compounds are effective as stereospecific catalysts towards oxidation, reduction, hydrolysis, biological activity and other transformations of organic and inorganic chemistry[24]. In organic compounds the presence of >C=N- along with other functional groups form more stable complexes compared to compounds with only >C=N- coordinating moiety.

Hence, coumarins containing a Schiff base are expected to have enhanced antitumor and other biological activities. It is well established that the biological activity associated with the hydrazone compounds is attributed to the presence of the active pharmacophore (-CONH-N=C-). Therefore, many hydrazone compounds containing this active moiety show good anticancer bioactivities[25].

For this study, aimed at extending the applications of coumarins, a number of simple substituted coumarin derivatives such as 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy]-N'-(substituted methylene)acetohydrazides (**4a-l**), expected to be biologically active, were synthesized and their chemical structures confirmed by means of FT-IR, ^{1}H NMR and mass spectral data[26]. This paper reports the antimicrobial, antioxidant activity studies and spectrophotometric results of these unsymmetrical Schiff bases of 2'-[(4-methyl-2-oxo-2-chromen-7-yl)oxy]acetohydrazides (**3**) when condensed using different aryl and heterocyclic aromatic aldehydes.

EXPERIMENTAL

Infrared (IR) spectra were recorded at room temperature from 4000-400 cm^{-1} with KBr pellets, using Avatar 330 equipped with DTGS detector. The ^1H NMR was measured on a Bruker AMX-400 instrument at room temperature using the X-WIN NMR version X-WIN NMR 1.3 cn drx software. The ^1H NMR was measured for *ca.* 0.03 M solutions in DMSO-d_6 using TMS as internal reference. Mass spectra were obtained using LC-MS-MS (3200 Q-trap). Melting points were determined in open capillaries and are uncorrected. All reagents were purchased from Aldrich and Qualigens and used without further purification.

General procedure: Procedure for the preparation of ethyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy] acetate (**2**). To a solution of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**) in dry DMF, anhydrous potassium carbonate (1.0 molar equiv) and ethyl chloro acetate (1.0 molar equiv) were added. The resultant mixture was stirred at 80 ºC for 10 h, cooled and then the reaction mixture was added to a large amount of water. The solid separated was filtered, washed with excess of water. The crude product was purified by crystallization from ethanol. The yield of product was 81-82 % [lit. yield 40 %][27]. m.p. 94-96 ºC [lit. 88-90 ºC][27]; IR (KBr, ν_{max}, cm^{-1}): 2999, 2917, 1765, 1724, 1615, 1391, 1196, 1508, 1476, 1196, 1080; ^1H NMR (400 MHz, DMSO, ppm), δ = 7.69 (1H, d, *J* = 9.5 Hz, 6-H), 6.99 (2H, m, 5, 8-H), 6.23 (1H, s, 3-H), 4.93 (2H, s, OCH_2), 4.18 (2H, q, *J* = 7.0 Hz, CH_2), 2.39 (3H, s, CH_3), 1.22 (3H, t, *J* = 7.0 Hz, CH_3); LC-MS: m/z 263.0 (M + 1).

Procedure for the preparation of 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetohydrazide (3): Ethyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]acetate (3, 0.01 mol) in ethanol (20 mL) was stirred at room temperature for 20 min. To this mixture hydrazine hydrate (0.014 mol) was added. The resultant mixture was stirred at room temperature for 15 min and the solid filtered with a sintered glass funnel. The residue was dried and then desiccated to afford a crystalline powder. The powder was recrystallized from chloroform/methanol and gave colourless needles. The yield of product was 88-90 %. m.p. 202-204 ºC [lit. 198-200 ºC][27]; IR (KBr, ν_{max}, cm^{-1}): 3331, 3268, 3082, 2958, 1731, 1676, 1609, 1508, 1439, 1153, 1074; ^1H NMR (400 MHz, DMSO, ppm), δ = 9.41 (1H, s, NH), 7.69 (1H, d, *J* = 8.8 Hz, 6-H), 7.00 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 5-H), 6.9 (1H, d, *J* = 10.3 Hz, 8-H), 6.21 (1H, s, 3-H), 4.61 (2H, s, NH_2), 4.35 (2H, s, OCH_2), 2.39 (3H, s, CH_3); LC-MS: m/z 249.0 (M + 1).

Procedure for the synthesis of 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(substituted methylene)acetohydrazides (4a-l): A mixture of compound **3** (0.01 mol) in chloroform/methanol (1:1) mixture (30 mL), aryl/hetero aromatic aldehyde (0.01 mol) and 1 mL of glacial acetic acid was refluxed on a water bath for 60-150 min. The mixture was allowed to cool and then the separated solid was filtered, washed with excess of methanol. The physical characteristics data of the synthesized compounds **4a-l** are given in Table-1.

Spectral data

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(benzylidene)acetohydrazide (4a): IR (KBr, ν_{max}, cm^{-1}): 3072, 2969, 1712, 1686, 1615, 1511, 1435, 1393, 1271, 1160, 1084; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.66 (1H, d, *J* = 7.5Hz, HC=N), 8.32 and 8.02 (1H, 2s, NH), 7.72 (1H, s, 6-H), 7.43 (5H, s, Ph-H), 7.04 (2H, d, *J* = 18.7 Hz, 5, 8-H), 6.23 (1H, d, *J* = 8.2 Hz, 3-H), 5.30 (1H, s, OCH_2), 4.81 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 335 (M - 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(4-hydroxybenzylidene)acetohydrazide (4b): IR (KBr, ν_{max}, cm^{-1}): 3280,3079, 2908, 1717, 1670, 1610, 1512, 1423, 1390, 1356, 1260, 1145, 1086; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.43 (1H, d, *J* = 15.9 Hz, HC=N), 9.90 (1H, s, OH), 8.21 and 7.91 (1H, 2s, NH), 7.71 (1H, d, *J* = 8.7Hz, 6-H), 7.63 (2H, d, *J* = 8.4Hz, 3', 5'-H), 7.00 (2H, m, 5, 8-H), 6.81 (2H, d, *J* = 8.4 Hz, 2',6'-H), 6.22 (1H, d, *J* = 7.7 Hz, 3-H), 5.25 (1H, s, OCH_2), 4.77 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 353 (M + 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(3-nitrobenzylidene)acetohydrazide (4c): IR (KBr, ν_{max}, cm^{-1}): 3073, 2958, 1720, 1687, 1617, 1529, 1433, 1387, 1347, 1275, 1138, 1085; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.88 (1H, s, HC=N), 8.52 and 8.45 (1H, 2s, NH), 8.25 (1H, d, *J* = 5.8 Hz, 6'-H), 8.21 (1H, d, *J* = 7.7Hz, 4'-H), 8.15 (1H, s, 2'-H), 7.8 (1H, t, *J* = 5.5 Hz, 5'-H), 7.71 (1H, d, *J* = 9.0 Hz, 6-H), 7.01 (2H, d, *J* = 10.3 Hz, 5, 8-H), 5.36 (1H, s, OCH_2), 4.85 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 382 (M + 1).

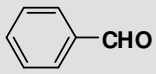
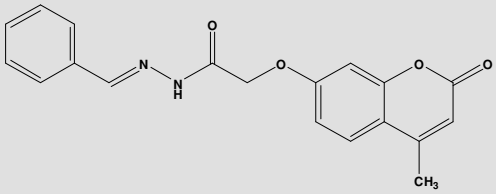
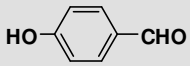
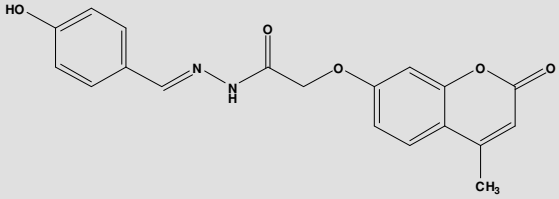
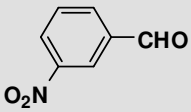
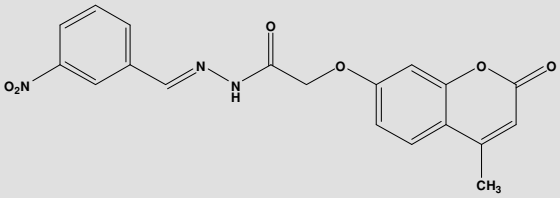
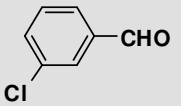
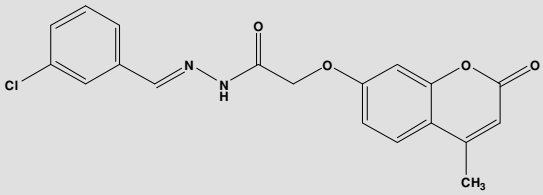
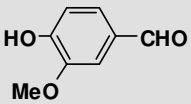
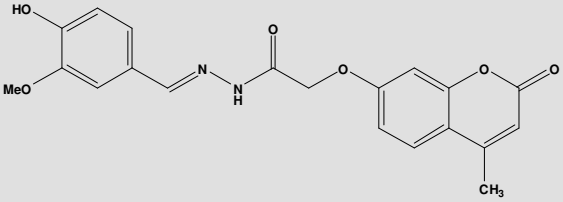
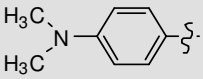
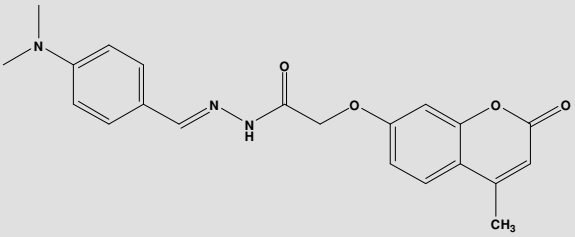
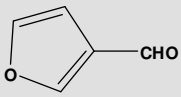
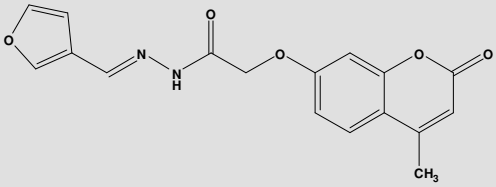
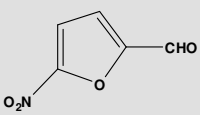
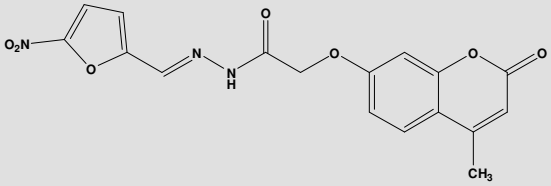
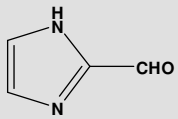
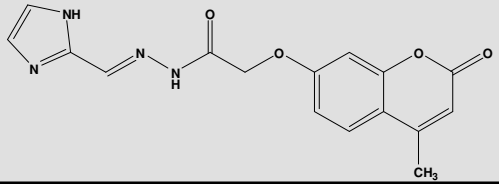
2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(3-chlorobenzylidene)acetohydrazide (4d): IR (KBr, ν_{max}, cm^{-1}): 3101, 2976, 1713, 1682, 1617, 1510, 1433, 1390, 1273, 1157, 1083; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.76 (1H, s, HC=N), 8.30 and 8.0 (1H, 2s, NH), 7.75 (1H, t, 5'-H), 7.71 (1H, d, *J* = 8.7Hz, 6-H), 7.5 (2H, t, *J* = 4.9Hz, 4', 6'-H), 7.02 (2H, m, 5, 8-H), 6.23 (1H, d, *J* = 8.9Hz, 3-H), 5.32 (1H, s, OCH_2), 4.82 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 371 (M + 1).

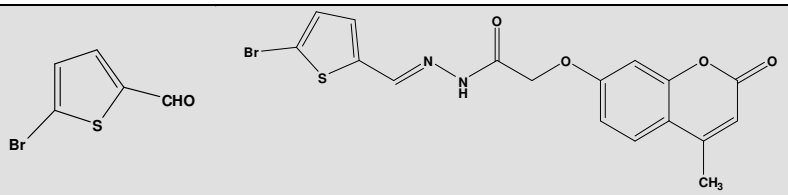
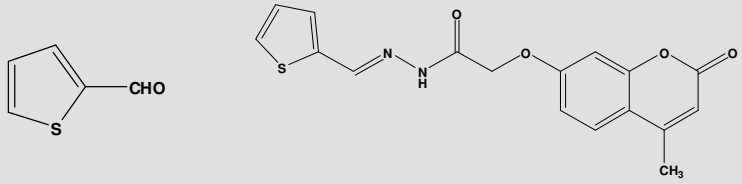
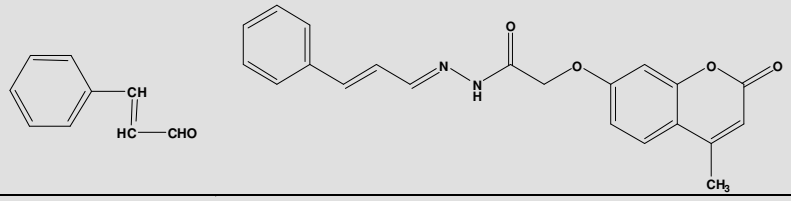
2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(4-hydroxy-3-methoxybenzylidene)aceto hydrazide (4e): IR (KBr, ν_{max}, cm^{-1}): 3482, 3135, 3086, 2975, 1710, 1697, 1612, 1518, 1428, 1390, 1267, 1161, 1077; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.46 (1H, d, *J* = 23.8 Hz, HC=N), 9.50 (1H, s, OH), 8.19 and 7.90 (1H, 2s, NH), 7.71 (1H, d, *J* = 7.8Hz, 6-H), 7.27 (1H, d, *J* = 9.9 Hz, 8-H), 6.98 (3H, m, 2', 5', 6'-H), 6.82 (1H, dd, *J* = 2.8 Hz, *J* = 8.1 Hz, 5-H), 6.22 (1H, d, *J* = 8.8 Hz, 3-H), 5.28 (1H, s, OCH_2), 4.78 (1H, s, OCH_2), 3.80 (3H, s, OCH_3), 2.40 (3H, s, CH_3); LC-MS: m/z 383 (M + 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(4-dimethylaminobenzylidene)aceto hydrazide (4f): IR (KBr, ν_{max}, cm^{-1}): 3316, 2913, 1706, 1683, 1615, 1525, 1444, 1366, 1270, 1152, 1081; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.36 (1H, d, *J* = 20.9 Hz, HC=N), 8.16 and 8.31 (1H, 2s, NH), 7.71 (1H, d, *J* = 4.8 Hz, 6-H), 7.51 (2H, d, *J* = 5.3 Hz, 3', 5'-H), 7.0 (2H, m, 5, 8-H), 6.73 (2H, d, *J* = 5.3 Hz, 2',6'-H), 6.22 (1H, d, *J* = 8.2 Hz, 3-H), 5.24 (1H, s, OCH_2), 4.76 (1H, s, OCH_2), 2.96 (6H, s, $N(CH_3)_2$), 2.40 (3H, s, CH_3); LC-MS: m/z 378 (M - 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-[(furan-3-yl)methylene]acetohydrazide (4g): IR (KBr, ν_{max}, cm^{-1}) : 3110, 2958, 1710, 1688, 1617, 1511, 1433, 1390, 1272, 1158, 1086; ^1H NMR (400 MHz, DMSO, ppm), δ = 11.52 (1H, d, *J* = 21.7 Hz, HC=N), 8.28 and 8.13 (1H, 2s, NH), 7.97 (1H, s, 2'-H), 7.74 (1H, s, 5'-H), 7.71 (1H, d, *J* = 8.7Hz, 6-H), 7.00

TABLE-1
PHYSICAL DATA OF THE COMPOUNDS **4a-l**

Entry	Reactant (R)	Product	Time (min)	Yield* (%)	m.p. (ºC)
4a			60	86.2	276-278
4b			60	84.3	280-282
4c			90	81.6	266-268
4d			60	83.2	266-268
4e			120	76.8	258-260
4f			120	69.7	260-262
4g			120	66.1	272-274
4h			120	64.8	284-286
4i			150	61.2	256-258

4j		60	89.4	288-290
4k		60	86.1	274-276
4l		120	72.6	270-272

*Isolated yields.

(1H, d, *J* = 2.4 Hz, 8-H), 6.99 (1H, d, *J* = 2.4 Hz, 5-H), 6.96 (1H, dd, *J* = 2.3 Hz, *J* = 9.1 Hz, 4'-H), 6.23 (1H, d, *J* = 8.5 Hz, 3-H), 5.22 (1H, s, OCH_2), 4.77 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 327 (M + 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-[(5-nitrofuran-2-yl)methylene]aceto hydrazide (4h): IR (KBr, ν_{max}, cm^{-1}): 3112, 1715, 1701, 1679, 1614, 1500, 1456, 1390, 1351, 1260, 1137, 1081; ^{1}H NMR (400 MHz, DMSO, ppm), δ = 12.03 (1H, d, *J* = 10.0 Hz, HC=N), 8.30 and 7.97 (1H, 2s, NH), 7.79 (1H, dd, *J* = 3.8 Hz, *J* = 10.1 Hz, 4'-H), 7.71 (1H, d, *J* = 8.6 Hz, 6-H), 7.28 (1H, dd, *J* = 3.8 Hz, *J* = 18.9 Hz, 3'-H), 7.00 (2H, m, 5, 8-H), 6.23 (1H, d, *J* = 8.8 Hz, 3-H), 5.28 (1H, s, OCH_2), 4.86 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 372 (M + 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-[(1*H*-imidazol-2-yl)methylene]acetohydrazide (4i): IR (KBr, ν_{max}, cm^{-1}): 3316, 3126, 3030, 2915, 1715, 1683, 1626, 1536, 1446, 1386, 1257, 1150, 1087; ^{1}H NMR (400 MHz, DMSO, ppm), δ = 12.60 (1H, s, NH), 11.65 (1H, d, *J* = 10.2 Hz, HC=N), 8.23 and 7.89 (1H, 2s, NH), 7.71 (1H, d, *J* = 3.4 Hz, 6-H), 7.34 (1H, s, 4'-H), 7.07 (1H, s, 5'-H), 7.03 (1H, d, *J* = 2.3 Hz, 8-H), 7.00 (1H, d, *J* = 2.2 Hz, 5-H), 6.23 (1H, d, *J* = 5.8 Hz, 3-H), 5.26 (1H, s, OCH_2), 4.82 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 327 (M + 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-[(5-bromothiophen-2-yl)methylene]aceto hydrazide (4j): IR (KBr, ν_{max}, cm^{-1}): 3053, 2917, 1713,1681, 1616, 1510, 1429, 1389, 1274, 1139, 1083; ^{1}H NMR (400 MHz, DMSO, ppm), δ = 11.68 (1H, d, *J* = 21.8 Hz, HC=N), 8.47 and 8.10 (1H, 2s, NH), 7.71 (1H, d, *J* = 8.7 Hz, 6-H), 7.28 (2H, m, 3',4'-H), 6.99 (2H, m, 5, 8-H), 6.23 (1H, d, *J* = 8.1Hz, 3-H), 5.18 (1H, s, OCH_2), 4.78 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 320 (M - 1), 322 (M + 1).

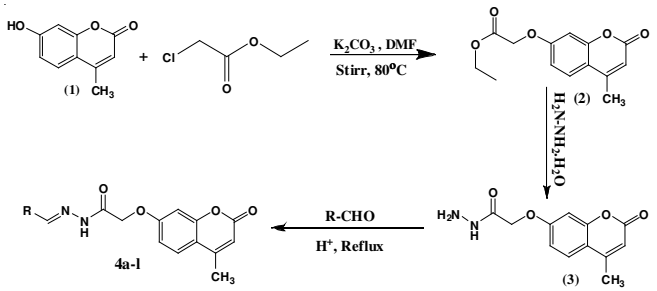
2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-[(thiophen-2-yl)methylene]acetohydrazide (4k): IR (KBr, ν_{max}, cm^{-1}): 3055, 2917, 1732, 1685, 1618, 1513, 1432, 1390, 1272, 1143, 1085; ^{1}H NMR (400 MHz, DMSO, ppm), δ = 11.62 (1H, d, *J* = 21.8 Hz, HC=N), 8.31 and 8.20 (1H, 2s, NH), 7.71 (1H, d, *J* = 5.8 Hz, 6-H), 7.65 (1H, d, *J* = 8.0 Hz, 5'-H), 7.47 (1H, s, 3'-H), 7.13 (1H, m, 4'-H), 7.00 (2H, m, 5,8-H), 6.23 (1H, d, *J* = 7.7 Hz, 3-H), 5.20 (1H, s, OCH_2), 4.79 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 341 (M - 1).

2-[(4-Methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(3-phenylallylidene)acetohydrazide (4l): IR (KBr, ν_{max}, cm^{-1}): 3313, 3043, 2914, 1704, 1685, 1625, 1533, 1421, 1390, 1251, 1151, 1084; ^{1}H NMR (400 MHz, DMSO, ppm), δ = 11.54 (1H, d, *J* = 15.3 Hz, HC=N), 8.09 and 7.84 (1H, 2d, *J* = 8.8 Hz, NH), 7.70 (1H, d, *J* = 8.8 Hz, 6-H), 7.61 (2H, d, 2', 6'-H), 7.38 (2H, t, *J* = 7.2 Hz, 4'-H), 7.09 (4H, m, 5, 8-H, HC=CH), 6.22 (1H, d, *J* = 7.8 Hz, 3-H), 5.18 (1H, s, OCH_2), 4.78 (1H, s, OCH_2), 2.40 (3H, s, CH_3); LC-MS: m/z 363 (M + 1).

RESULTS AND DISCUSSION

Resorcinol treated with ethyl acetoacetate in presence of sulphuric acid at 10 ºC yielded 7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**)[28]. Treating compound **1** in dry DMF with ethyl chloroacetate in the presence of K_2CO_3 at 80 ºC for 10 h yielded ethyl 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-acetate (**2**) in 82 % yield, whereas in literature the conversion of **1** into **2** was reported under the refluxing conditions for 10 h in dry acetone[29-33] with 40 % yield. Compound **2** was heated with hydrazine hydrate in ethanol on a water bath for 1 h to obtain 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-acetohydrazide (**3**). The 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(substituted methylene)acetohydrazides (**4a-l**) synthesized by the condensation of 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy] acetohydrazide (**3**) with different aryl/hetero aromatic aldehydes and characterized through the spectral data (**Scheme-I**).

Biological study: All of the synthesized substituted coumarin derivatives were screened for their *in vitro* antimicrobial activity against standard pathological bacterial strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25619 and *Staphylococcus aureus* ATCC 25923 at 1.0, 0.5, 0.25 mg/mL concentration in Mueller-Hinton agar medium by agar well diffusion method. Tetracycline was used as standard drugs for antibacterial activity, respectively. The minimal inhibitory concentration (MICs, mg/mL) results of the *in vitro* assays of anti-



Scheme-I: Synthetic protocol of compounds **4a-l**

bacterial activity of all the synthesized compounds **4a-l** are shown in Table-2. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of each one of the compounds that inhibited visible growth. It was confirmed that the solvent had no antimicrobial activity against any of the test microorganisms.

TABLE-2
ANTIBACTERIAL ACTIVITY OF COMPOUNDS, **4a-l**

Compound	Antibacterial activity* (MIC, mg/mL)		
	S. aureus	*E. coli*	*P. aeruginosa*
4a	13 (1.0)	10 (0.5)	9 (1.0)
4b	12 (0.5)	9 (0.25)	8 (1.0)
4c	9 (0.5)	12 (1.0)	9 (1.0)
4d	11 (1.0)	9 (0.25)	8 (0.5)
4e	10 (0.25)	11 (0.5)	10 (1.0)
4f	9 (0.25)	12 (0.5)	8 (0.25)
4g	12 (1.0)	10 (0.5)	11 (1.0)
4h	8 (0.25)	9 (0.5)	8 (0.25)
4i	13 (1.0)	8 (0.25)	12 (1.0)
4j	9 (0.25)	11 (1.0)	12 (1.0)
4k	11 (0.5)	14 (1.0)	10 (1.0)
4l	14 (1.0)	10 (0.5)	11 (1.0)
Tetracycline**	19 (1.0)	21 (1.0)	12 (1.0)

*Zone of inhibition (mm); **Standard antibacterial drug.

Antibacterial activity: According to preliminary antibacterial screening by well diffusion method, compounds **4a-l** were found to be active against all the three standard bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The compounds **4e**, **4f**, **4h** and **4j** exhibited a MIC of 0.25 mg/mL against *Staphylococcus aureus*, whereas compounds **4b**, **4d** and **4i** exhibited significant antibacterial activity (MIC 0.25 mg/mL) against *Escherichia coli*. The compounds **4f**, **4h** and **4d** exhibited promising antibacterial activity (MIC 0.25, 0.25 and 0.5 mg/mL) against *Pseudomonas aeruginosa*, whereas the compounds **4a**, **4e-h** and **4l** exhibited a MIC of 0.5 mg/mL against *Escherichia coli*. The compounds **4b**, **4c** and **4k** showed good antibacterial activity at a MIC of 0.5 mg/mL against *Staphylococcus aureus*. The compounds **4a**, **4d**, **4g**, **4i**, **4l** and **4c**, **4j**, **4k** exhibited antibacterial activity (MIC 1.0 mg/mL) against *Staphylococcus aureus* and *Escherichia coli*, respectively whereas the compounds **4a-c**, **4e**, **4g** and **4i-l** exhibited activity (MIC 1.0 mg/mL) against *Pseudomonas aeruginosa*. The antibacterial activity results of all the synthesized comp-ounds are given in Table-2 and compared with standard tetracycline.

Antioxidant activity: All the synthesized compounds **4a-l** were subjected to screening for their possible antioxidant activity using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and the results are given in the Table-3. To study the radical scavenging effects of all the compounds **4a-l**, DPPH was used which is a stable free radical with a characteristic absorption at 517 nm.

TABLE-3
ANTIOXIDANT ACTIVITY OF COMPOUNDS **4a-l** AT 0.1 mg CONCENTRATION USING DPPH METHOD*,**

After 0.5 h incubation					
Entry	Absorbance	Antioxidant activity (%)	Entry	Absorbance	Antioxidant activity (%)
4a	0.626	2.49	**4g**	0.597	7.00
4b	0.587	8.56	**4h**	0.605	5.76
4c	0.590	8.09	**4i**	0.583	9.19
4d	0.583	9.19	**4j**	0.620	3.42
4e	0.029	95.48	**4k**	0.636	0.93
4f	0.555	13.55	**4l**	0.620	3.42
Control	0.642	–	–	–	–

*Test sample concentration = 0.1 mg in 100 µL of DMSO; OD values observed at 517 nm. **Ascorbic acid was used as a standard antioxidant.

As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. The degree of discolouration indicates the scavenging potential of the antioxidant. At 0.1 mg concentration, compound **4e** and ascorbic acid exhibited 95.48 and 100 % free radical scavenging activity, respectively, by this method.

According to preliminary antioxidant screening by the DPPH radical scavenging method, compounds (**4a-l**) were found to be active against the diphenylpicrylhydrazyl radicals. Of the compounds **4a-l**, the compound **4e** at a concentration of 0.1 mg exhibited the highest radical scavenging activity of 95.48 % and the least of 0.93 % by the compound **4k**. The maximum antioxidant activity was exhibited by compound **4e** (95.48 %), having 3-methoxy-4-hydroxybenzylidene ring as substitute R and the minimum antioxidant activity was exhibited by compound **4k** (0.93 %), having 2-thiophenyl ring as substitute R. The percentage of antioxidant activity by the compounds **4a-l** was given in Fig. 1 as a graphical representation. At different concentrations of compound **4e**, antioxidant activity was determined by comparing with ascorbic acid at the same concentrations, the results are given in Table-4 and represented as a graphical format in Fig. 2.

The free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)[34]. In brief, each compound (500 µL) containing 0.1 mg of soluble solids/mL were added to a DMSO solution of DPPH (0.1 mmol, 3 mL). After a 0.5 h incubation period at ambient temperature in dark, the absorbance was read at 517 nm. The controls contained all the reaction reagents except the synthetic compound or positive control substance. The experiment was carried out in triplicate. The DPPH scavenging activity was expressed as the inhibition of free radical DPPH in per cent (I %) as described by Tepe *et al.*[35].

$$\text{Inhibition } (\%) = \left[\frac{A_{Blank} - A_{Sample}}{A_{Blank}}\right] \times 100$$

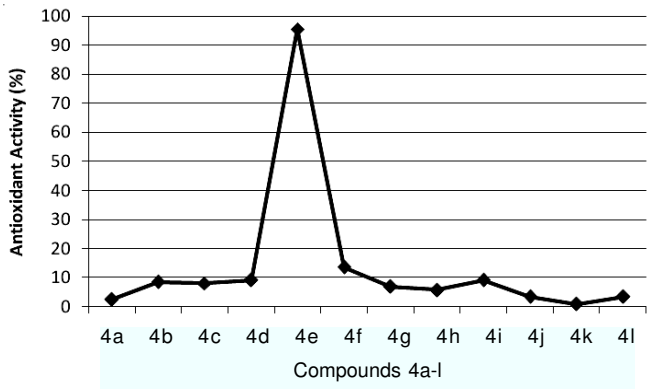


Fig. 1. Antioxidant activity of compounds **4a-l** using DPPH radical scavenging method at 0.1 mg concentration

TABLE-4
ANTIOXIDANT ACTIVITY OF COMPOUND **4e** AT DIFFERENT CONCENTRATIONS USING DPPH RADICAL SCAVENGING METHOD*

After 0.5 h incubation					
Compound **4e** (µg)	Absorbance	Antioxidant activity (%)	Ascorbic acid (µg)	Absorbance	Antioxidant activity (%)
100	0.029	95.48	100	0.000	100.00
75	0.054	91.58	75	0.000	100.00
50	0.110	82.86	50	0.000	100.00
25	0.196	69.47	25	0.021	96.73
5	0.426	33.64	5	0.199	69.00
Control	0.642	–	–	–	–

*Solvent used is DMSO; OD values observed at 517 nm.

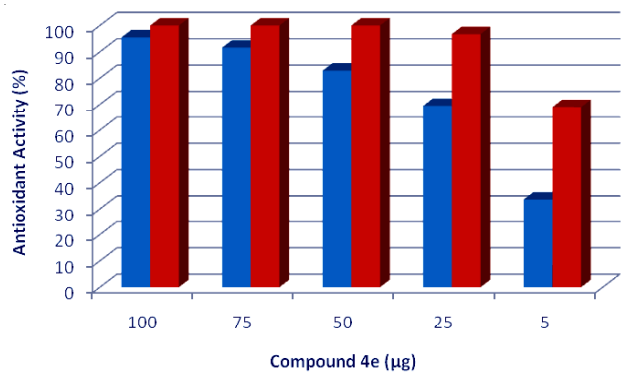


Fig. 2. Antioxidant activity of compound **4e** ■ and ascorbic acid ■ using DPPH radical scavenging method at different concentrations

Spectrophotometric determination: The presence of chromophores in a molecule is best documented by UV-visible spectroscopy. The absorbance of a sample will be proportional to the number of molecules (molar concentration in the sample tube) absorbing radiation. It is necessary to correct the absorbance value for this sample concentration and other operational factors if the spectra of different compounds are to be compared in a meaningful way. The corrected absorption value is called "molar absorptivity" and is particularly useful when comparing the spectra of different compounds and determining the relative strength of light absorbing functions (chromophores).

UV-Visible analysis and molar absorptivity, ε, which is the proportionality constant between the measured absorbance and the concentration for the absorbance band of interest, is recorded for all the synthesized compounds **4a-l** at a wavelength range of 200-700 nm. All the absorbance and molar absorptivity (ε) values at different wavelengths are given in Table-5. Based on the solubility of all the compounds, absorption values are recorded using DMSO as a solvent. The compound **4a** shows the maximum molar absorptivity ε = 81010, having phenyl ring as substitute R and the compound **4c**, having 3-nitrobenzylidene ring as substitute R, shows the minimum molar absorptivity, ε = 57. All these molar absorptivity (ε) values of compounds **4a-l** are useful for the identification of chromophores present in a molecule. Shimadzu UV-1700(E) 23 OCE model UV-visible spectrophotometer was used for the determination of absorbance values for all the synthesized compounds **1a-l**, at a wavelength range of 200-700 nm using DMSO as a solvent. The molar absorptivity (ε) values are calculated as per the standard formula mentioned below.

$$\text{Molar absorptivity } (\varepsilon) = \frac{A}{c \cdot b}$$

where A = absorbance, c = sample concentration in mol/L, b = length of light path through the cuvette in cm.

Conclusion

The modified procedure for the conversion of **1** into **2** with dry DMF at 80 ºC instead of dry acetone resulted with improved yield of 82 %. The new Schiff base 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy]-N'-(substituted methylene) acetohydrazides (**4a-l**) have been synthesized by reaction of 2-[(4-methyl-2-oxo-2*H*-chromen-7-yl)oxy] acetohydrazide (**3**) with aryl/hetero aromatic aldehydes under reflux temperature. The compounds (**4a-l**) were tested for antibacterial activity by agar well diffusion method, showing moderate to potent inhibition. This paper presents a method developed to determine the antioxidant activity of the synthesized compounds **4a-l** by DPPH radical scavenging method using DMSO as a solvent. The results obtained in the present study clearly demonstrate that the compound **4e** was effectively scavenging free

TABLE-5
UV AND MOLAR ABSORPTIVITY VALUES OF COMPOUNDS **4a-l** IN A WAVELENGTH RANGE OF 200-700 nm

At 0.02 mg concentration*							
Entry	UV-Visible analysis		Molar absorptivity (ε_{max})	Entry	UV-Visible analysis		Molar absorptivity (ε_{max})
	λ_{max}	Absorbance			λ_{max}	Absorbance	
4a	295	1.604	81010	**4g**	323	0.088	4314
4b	308	1.302	68889	**4h**	375	0.373	20722
4c	400	0.001	57	**4i**	313	0.642	31471
4d	313	0.212	11778	**4j**	325	0.609	29279
4e	322	0.526	30057	**4k**	318	1.191	61077
4f	347	0.651	36989	**4l**	320	1.457	79185

*DMSO is used as a solvent.

radicals under *in vitro* conditions using DPPH radical scavenging method. The UV-visible absorbance and molar absorptivity (ε) values at different wavelengths using 0.02 mg concentration are reported.

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