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Synthesis and anticancer activity of some new 2-[(4-methy-2-oxo-2H- chromen-7-yl)-oxy]acetamide derivatives

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ABSTRACT

*Newly designed2-[(4-methy-2-oxo-2H- chromen-7yl) oxy] acetamide derivatives (**4a-4g**) have been synthesized in good yields and characterized by advanced spectroscopic methods. The synthesized coumarinyloxyacetamide derivatives have been studied for their anticancer as well as antimicrobial activities.*

Keywords: Coumarinyloxy acetamides, anticancer activity.

INTRODUCTION

Coumarinheterocycle is one of the major classes of naturally occurring flavanoidor chromen compounds. Coumarin derivatives exhibit wide range of biological activities such as antimicrobial [1,2], anticoagulant [3], antiallergic, anticancer [4], anti-inflammatory [5], antioxidant [6,7] and calcium channel blocking activity [8]. Because of variety of biological activities, both synthetic and naturally occurring coumarin derivatives have been widely studied all over the world. Recently 3-amino coumarin derivatives have been reported as DPP-IV inhibitors from our laboratory [9].

Canceris fatal disease after cardiovascular in terms of morbidity and mortality affecting human health worldwide [10].On other side, multidrug resistance is an increasing concern with current antibacterial agents for treatment of infectious diseases [11]. The coumarincontaining antibiotic, novobiocin is active against grampositive bacteria as potent inhibitor of DNA replication [12].

From our laboratory we have reported amide derivatives of benzodifuran-2-carboxylic acid [13] as antimicrobial agents. We have also reported coumarin derivatives as anticancer agents [14]. It prompted us to combine amide moiety with coumarin derivatives, so that it may show antimicrobial as well as anticancer activity. Thus in continuation of our work on search of new coumarin derivatives, we report herein synthesis characterization and antibacterial as well as anticancer activity of coumarinyloxy amide derivatives.

MATERIALS AND METHODS

Experimental

Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus.IR spectra were recorded as KBr disc on Perkin Elmer RX-1 spectrophotometer.^{1}H NMR and ^{13}C NMR spectral data

were recorded from a Brucker Advance 400 spectrometer (400MHz). TLC was performed on silica gel F 254 plates (Merck).CHN elemental analyses were recorded on Eager Scientific AnalyticalInstrument.

Chemistry

The starting compound 7-hydroxy-4-methyl -2H-chromen-2-one was preparedby the reported method [15].

Synthesis of Ethyl-2-(4-methyl-2-oxo-2H-chromen-7-yl) oxy] acetate (2)

To the stirred solution of **1** (1 g, 5.0mmol, 1.0 eq) in DMF, ethylchloroacetate (0.9 g, 5 mmol, 1.2 eq) and K_2CO_3 (0.9 g, 6.5 mmol, 1.3eq) were added and the resulting mixture was refluxed for 16 h.After completion of reaction (monitored by TLC), reaction mixture was cooled to room temperature and poured on to crushed ice to give solid. The solid was filtered, washed with water and recrystallized from ethanol to give compound **2**. Yield85 %;m.p: 110-112°C; IR (KBr): 3085, 2950, 2880, 1750, 1705, 1615, 1375, 1210, 950 cm^{-1};^{1}H-NMR($CDCl_3$, 400MHz) δ1.33 (3H, d, *J* = 7.2 Hz), 2.41 (3H, s), 4.30(2H, q, *J* = 7.2 Hz), 4.70 (2H, s), 6.17 (1H, d, *J* = 1.2 Hz), 6.78 (1H, d, *J* = 2.8 Hz), 6.93 (1H, dd, *J* = 8.8, 2.8 Hz), 7.54 (1H, d, *J* = 8.8 Hz); X-ray crystal data (CCDC No. 1000266817) is given in Table-1

Synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid (3)

Compound **2** (2.62 g, 10.0 mmol, 1.0eq) was treated with KOH (5.6 g, 100.0 mmol, 10.0eq) in Ethanol (30 ml). The resulting mixture wasrefluxed at a temperature 100–108 °C for 15 h. After that the reaction mixture was allowed to cool up toroom temperature, the resulting residue waspoured into ice cold water and acidified with Con. HCl to pH 2. The resulting solid was filtered off and washed with cold water. The solid was dissolved in saturated $NaHCO_3$ solution, acidified with conc.HCl to give white solid. The solid was filtered, washed with water, dried and recrystallized from ethanol to give compound **3** as a white solid. % Yield 95%, m.p : 246-248 °C; ^{1}H-NMR (DMSO-d_6, 400MHz) δ2.37 (3H, s), 4.82 (2H, s), 6.20 (1H, s), 6.94 (2H, s), 7.66 (1H, d, *J* = 8.4 Hz); ^{13}C-NMR (DMSO-d_6, 100 MHz)δ18.57, 65.23, 101.89, 111.80, 112.73, 113.95, 126.94, 153.85, 154.95, 160.59, 161.20, 170.11.

General procedure for the synthesis of amides (4a-4g):

A suspension of compound **3** (0.5 g, 2.136mmol) in dichloromethane(DCM) (25 mL) was cooled to 0-5°C. To this oxylyl chloride (0.46 mL, 5.34 mmol, 2.5 eq) was added drop wise at 0-5°C followed by a drop of DMF. The resulting solution was stirred at 0-5°C for30min and at RT for 3 h. The resulting solution was concentrated on rotavapor to give residue. The residue was taken in DCM and concentrated to remove traces of oxylyl chloride. The residue was dissolved in DCM (25 mL) and cooled to 0-5°C. To this cold solution, different amines (1.1 eq.) were added followed by the (TEA)triethylamine (1.5 eq). The resulting mixture was stirred at 0-5°Cfor 30 min and then at RT for the 16 h. The reaction mixture was washed with water (25 mL), sat. $NaHCO_3$(25 mL) and then brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give crude compound.Thecrude product was purified by column chromatography (60–120 mesh) using Pet. ether: EtOAc (70:30 to 20:80) to give corresponding amide (**4a-4g**) in good to excellent yield.

Spectral data:

4-methyl-7-(2-oxo-2-pyrrolidin-1-ylethoxy)-2*H*-chromen-2-one (4a)

Yield : 82 %; off white solid (EtOH); m.p°C: 160–162 °C;IR (KBr): 3063, 2973, 2875, 1724, 1656, 1612, 1559, 1437, 1331, 1264, 1199, 1155, 1081, 977, 871, 799 cm^{-1};^{1}H-NMR ($CDCl_3$, 400MHz)δ d 1.86–1.92 (2H, m), 2.06–1.99 (2H, m), 3.55-3.49 (4H, m), 4.70 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.80 (1H, d, *J* = 2.4 Hz), 6.96 (1H, dd, *J* = 8.8, 2.4 Hz), 7.51 (1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.68, 23.82, 26.24, 26.48, 67.36, 101.81, 101.91, 112.28, 112.51, 112.59, 114.23, 125.75, 152.52, 155.05, 161.00 161.16, 165.34; MASS: $[M+H]^+$ 288.10; Elemental Analysis for $C_{17}H_{19}NO_4$ Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 66.89;H 5.96 ;N 4.88

4-methyl-7-(2-oxo-2-piperidin-1-yl-ethoxy)-2*H*-chromen-2-one (4b)

Yield : 85 %; off white solid (EtOH); m.p°C: 172–174 °C;IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};^{1}H-NMR ($CDCl_3$, 400MHz)δ1.57–1.69 (6H, m), 2.38(3H, s), 3.44–3.73 (4H, m), 4.77 (2H, s), 6.14 (1H, d, *J* = 1.2 Hz), 6.81(1H, d, *J* = 2.4 Hz), 6.94 (1H, dd, *J* = 8.8, 2.4 Hz), 7.50(1H, d, *J* = 8.8 Hz);^{13}C-NMR ($CDCl_3$, 100 MHz) δ18.70, 24.37, 25.48, 26.48, 43.24, 46.20, 67.16, 101.97, 112.29,112.39, 114.22, 125.77, 152.52, 155.03, 161.02, 161.18, 164.98; Elemental Analysis for $C_{17}H_{19}NO_4$ Calculated,%:C 67.76;H 6.36;N 4.65,Found,% C 63.36;H 5.65;N 4.61.

4-methyl-7-(2-morpholin-4-yl-2-oxo-ethoxy)-2*H*-chromen-2-one (4c)
Yield: 85 %; white solid (EtOH);m.p:146–148 °C; IR (KBr): 3069, 2993, 2939, 2859, 1725, 1663, 1610, 1502, 1450, 1429, 1390, 1255, 1196, 1084, 1012, 973, 858 cm^{-1};^{1}H-NMR (DMSO-d_6, 400MHz)δ d2.39 (3H, S), 3.36 (4H, br s), 3.76 (4H, br s), 4.95 (2H, s), 6.22 (1H, s), 6.98 (2H, br s), 7.68 (1H, d, *J* = 9.2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.58, 31.14, 52.40, 65.31, 101.99, 111.95, 112.77, 114.14, 127.00, 153.79, 154.98, 160.51, 161.00, 169.16; Elemental Analysis for $C_{16}H_{17}NO_5$:Calculated,%:C 63.36;H 5.65;N 4.62,Found,% C 67.76;H 6.36;N 4.61.

4-methyl-7-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-2*H*-chromen-2-one (4d)
Yield: 68 %; Off white solid (EtOH); m.p: 98-100 °C;IR (KBr):3476, 3419, 3069, 2924, 2806, 1716, 1644, 1610, 1437, 1391, 1372, 1258, 1207, 1157, 1138, 1079, 861 cm^{-1};^{1}H-NMR (DMSO-d_6, 400MHz)δ 2.27 (3H, s), 2.39(4H, s), 2.5 (3H, s), 3.5 (4H, S), 4.99 (2H, s), 6.21 (1H, d, *J* = 1.2 Hz), 6.97-6.94 (2H, m), 7.67 (1H, dd, *J* = 9.2, 1,2 Hz);^{13}C-NMR (DMSO-d_6, 100 MHz) δ18.60, 41.23, 43.95, 45.66, 66.38, 101.94, 111.67, 113.06, 113.77, 126.79, 153.88, 155.01, 160.59, 161.66, 165.62; Elemental Analysis for $C_7H_{20}N_2O_4$ Calculated,%:C 64.54;H 6.37;N 8.86,Found,% C 64.54;H 6.37;N 8.86.

***N*-(4-methylphenyl)-2-[(4-methyl-2-oxo-2*H*-chromen-7-yl) oxy] acetamides (4e)**
Yield: 74%; off white solid (EtOH); m.p: 214-216 °C; IR (KBr): 3365, 3304, 3038, 2914, 1700, 1681, 1627, 1594, 1534, 1392, 1364, 1296, 1153, 1081, 887, 849, 819 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz)δδ 2.25 (3H, s), 2.40 (3H, s), 4.82 (2H, s), 6.23 (1H, s), 7.07-7.02 (2H, m), 7.11 (2H, d, *J* = 8.4 Hz), 7.51(2H, d, *J* = 8.4 Hz), 7.72 (1H, d, *J* = 8.8, Hz), 10.09 (1H, s); ^{13}C-NMR($CDCl_3$, 100 MHz): δ 18.61, 20.92, 67.74, 102.12, 111.90, 112.86, 114.08, 120.13, 127.02, 129.61, 133.19, 136.25, 153.84, 154.98, 160.53, 161.30, 166.05; Elemental Analysis for $C_{19}H_{17}NO_4$: Calculated,%:C 70.58;H 5.30;N 4.33,Found,% C 70.58;H 5.30;N 4.33.

Anticancer activity Method:
MTT assay
The compounds were tested for their cytotoxic potential on three types of cancer cells, *viz.*, A549 (lung cancer cell-line), MCF7 (breast cancer cell-line) and A375 (melanoma cell-line). The MTT assay was used to determine the effect of each compound on the proliferation of cancer cells.

A549, MCF7 and A375 cultures were purchased from National Centre for Cell Science, Pune, India. All growth media, supplements and reagents were purchased from HiMedia Labs, Mumbai, India. For the assay, cells were seeded at 10^5 cells/ml in a 96-well plate in dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). To each well, test compound was added at six different concentrations of 100µM, 50µM, 10µM, 5µM, 1µM and 0.5µM. Each concentration was tested in triplicates. The cells were incubated with these compounds at 37^{o}C under 5% CO_2 for 48 hours. Following this, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5mg/ml. Cells were incubated with this tetrazolium dye for 4 hours. Subsequently, purple crystals of formazan were observed in each well, formed as a metabolic product of MTT. These crystals were dissolved in Isopropanol and the absorbance in each well was recorded at 570nm in a microplate reader (MicrotekSigma360). Absorbance at 570nm directly correlates with cell viability. IC_{50} values were calculated from concentration-response data using Graph Pad Prism software. Results are shown in Table 2.

Antimicrobial activity:
All the synthesized compounds were tested for their antibacterial activity against Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, *BacillusSubtilis*) by cup plate method [16] at 100 ppmconcentration in DMF solvent. Ampicillin was used as standard drug. All compounds did not show activity against all types of Gram positive and Gram negative strains.

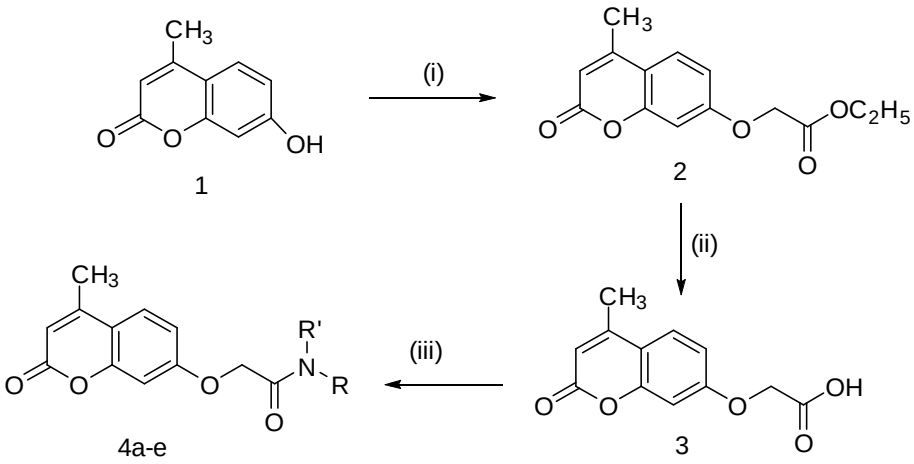
Antibacterial activity of all the synthesized compounds was tested in vitro by (cup plate method) serial agar dilution in which bacterial strains of Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, *Bacillus Subtilis*) were used, using serial agar dilution (cup plate method).The two microorganisms were cultured in petri dishes containing agar medium, cups (8 mm) were put onto the dishes and each synthesized compound dissolved in DMF (0.1 ml of 10 mg/ml) was added into the cups under aseptic condition. Then, the petri dishes were incubated at 37 ^{o}C for 24 h. The zone of inhibition of the growth of the bacteria, which were produced by diffusion of the compounds from the cup into the surrounding medium, was

measured to evaluate the antibacterial activity. Each experiment was repeated twice. DMF was used as a positive control for the experiments. Results are shown in Table 3.

RESULTS AND DISCUSSION

Chemistry

7-Hydroxy-4-methyl-benzopyran-2[H]-one (**1**) has been prepared by Pechmann condensation of resorcinol with ethyl acetoacetate using concentrated sulfuric acid. 7-Hydroxy-4-methyl coumarin (**1**), on reaction with ethyl chloro acetate in presence of anhydrous potassium carbonate and dry dimethyl formamide (DMF) gave 2-(4-methyl)-2-oxo-2[H]-chromen-7-yloxy) acetate **2**. The structure of **2** was proved from its IR, ^{1}H-NMR and ^{13}C-NMR. Moreover its single crystal was developed from methanol:Pet.ether by slow evaporation technique and proved the structure of **2 by** X-ray Single Crystal. Its CCDC no.is 1439494. It is observed that during single crystal formation transesterification has occurred, and ethyl ester get converted into methyl ester.



Reagents & Conditions: (i) $ClCH_2COOC_2H_5$, K_2CO_3, DMF reflux; (ii) Ethanolic KOH (15 %), reflux; (iii) (1) ClCOCOCl, DMC at 0-5°C, 30 min, RT,4 h; (2) RR'NH, TEA, DCM.

Scheme-1: Synthesis of carbyloxyacetamide derivatives of 4-methyl-7-hydroxy coumarin compounds

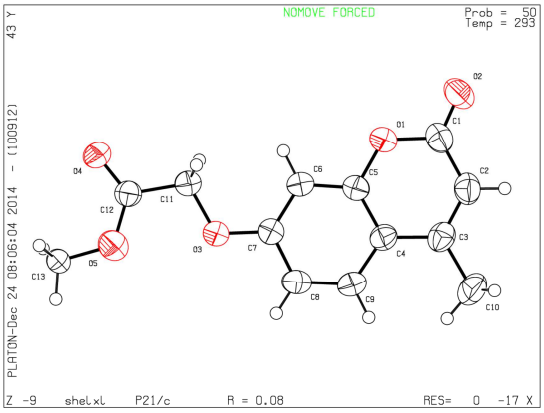


Figure 1: X-ray crystal structure of compound 2 as methyl ester

Table-1 Crystal data and structure refinement parameter for compound 2

Empirical formula	$C_{13}H_{12}O_5$
Formula Weight	248.23
Temperature/K	293(2)
Crystal system	Monoclinic
Space group	$P2_1/c$
a/ Å	10.0839(5)
b/ Å	14.5821(5)
c/ Å	8.4610(4)
α/°	90.00
β/°	112.403(6)
γ/°	90.00
Volume/Å3	1150.23/(9)
Z	4
$p_{Calc}^{mg/mm3}$	1.433
2Θ range for data collection	9.48 to 146.28°
Index range	$-11 \leq h \leq 12, -18 \leq k \leq 16, -10 \leq i \leq 10$
Reflections collected	5597
Independent reflections	2296[R(int)=0.0218]
Peak and hole e $Å^{-3}$	0.58/-0.49

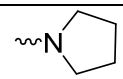
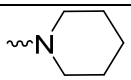
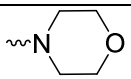
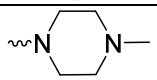
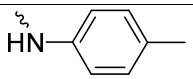
Then ester**2**was hydrolyzed by using aqueous KOH and then acidified withconc.HCl solution to give corresponding acid **3**. The obtained 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)acetic acid **3** was then converted into acid chloride in situ by the reaction with oxylyl chloride which was immediatelytreated with various amines (aromatic/aliphatic) to give corresponding 7-coumarinyloxyacetamides derivatives(**4a** - **4e**). All the synthesized compounds were purified by recrystallization from ethanol/column chromatography and were characterized by advanced spectral techniques.

The formation of coumarinyloxyacetamides was confirmed by IR spectroscopy. The formation of amide group was confirmed by the amide characteristic peak at 1680-1640 cm^{-1}corresponds to amide group and lactone carbonyl at 1700-1740cm^{-1} In^{1}H-NMR spectra, aromatic protons appeared in region of 6.12-7.68, the methylene group appeared in region of 4.70-4.95 and aliphatic protonsappeared in range of δ1.5-3.7.

Anticancer Activity

All the synthesized compounds were screened againstA549 (lung cancer cell-line) and one of the compound **4d** was screened against A375(melanoma cell-line). IC_{50}(μM) values were determined using Graph Pad prism softwarefor compounds **4a-e** as shown in Table 2.

Table 2:Anticancer activity (IC50, μg/mL) of substituted aminomethylnaphthopyrones4a-i

Compound	-NR^{1}R^2	IC_{50} (μM)[a]	
		A549	A375
4a		9.26	ND
4b		0.66	ND
4c		0.41	ND
4d		NA	0.041
4e		1.1nM	ND

[a]*IC_{50} values were determined using Graph Pad Prism software.*
NA= Not activeND = not determined

From the MTT assay, pyrrolidine compound **4a** showed better activity against A549 with IC_{50}value9.26 μM. On replacement of pyrrolidine with piperidine in compound **4b** resulted in compound with 14 fold higher activity

against A549 with IC_{50}value0.66 µM. Compound **4c** containing morpholine ring showed 22 fold higher activity compared to compound **4a** against A549 cell line with IC_{50}value0.41 µM. *N*-methyl piperazine compound **4d**did not show any activity against A549 cell line, but it showed very good activity against A375 cell line with IC_{50}value0.041µM. Interestingly, p-toluidine substituted compound **4e** showed excellent activity against A549 cell line with IC_{50}value1.1 nM.

Antimicrobial and antifungal activity

Table 3: Antimicrobial and antifungal activity of compounds4a-4e

Compounds	Gram –Ve bacteria		Gram +ve bacteria		Fungi
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus Subtilis*	*Candida Albicans*
4a	>228	>228	>228	>228	>228
4b	>228	>228	>228	>228	>228
4c	>228	>228	>228	>228	>228
4d	>228	>228	>228	>228	>228
4e	>228	>228	>228	>228	>228

Compounds **4a**-**4e** did not show any antimicrobial activity against tested gram –ve bacteria (*E. coli*, *P. aeruginosa)*and gram +ve bacteria (*S.aureus*, *B. subtilis)*. Also compounds **4a**-**4e** found inactive against fungi *C.Albicans*.

CONCLUSION

In conclusion, we have reported here synthesis of 7-coumarinyloxyacetamides derivatives(**4a** - **4e**) and their anticancer activity. Compounds **4a**-**4e** have shown promising anticancer activity against A549 (lung cancer cell-line). Compounds **4b** and **4c** are showing very good activity against A549 (lung cancer cell-line) with IC_{50} values 0.66 and 0.41 µM respectively. Compounds **4e** showed excellent activity against A549(lung cancer cell-line) with IC_{50} value1.1 nM. While compound **4d**is showing very good activity against A375 (melanoma cell-line) with IC_{50}value0.041 µM. Unfortunately all the synthesized compounds didn't show any antimicrobial activity.

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