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Review Article

Synthesis, Characterization and Anti-Cancer Activity of Some New Heterocycles Bearing Coumarin Moiety

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Abstract

8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde (1) was prepared and allowed to react with nucleophilic reagents to synthesize a series of some new heterocyclic compounds (2-20). All the new compounds have been characterized by IR, 1H-NMR spectral data. All the target compounds were evaluated for their *in vitro* anti-tumor activity against four cell lines: Human hepatocellular carcinoma (Hep-G2), colon carcinoma cells (HCT-116), breast adenocarcinoma (MCF-7) and prostate carcinoma (PC-3).

Keywords: Anticancer Activity; Heterocyclic compounds; Microwave Assistant; Multi-Component Reactions

Introduction

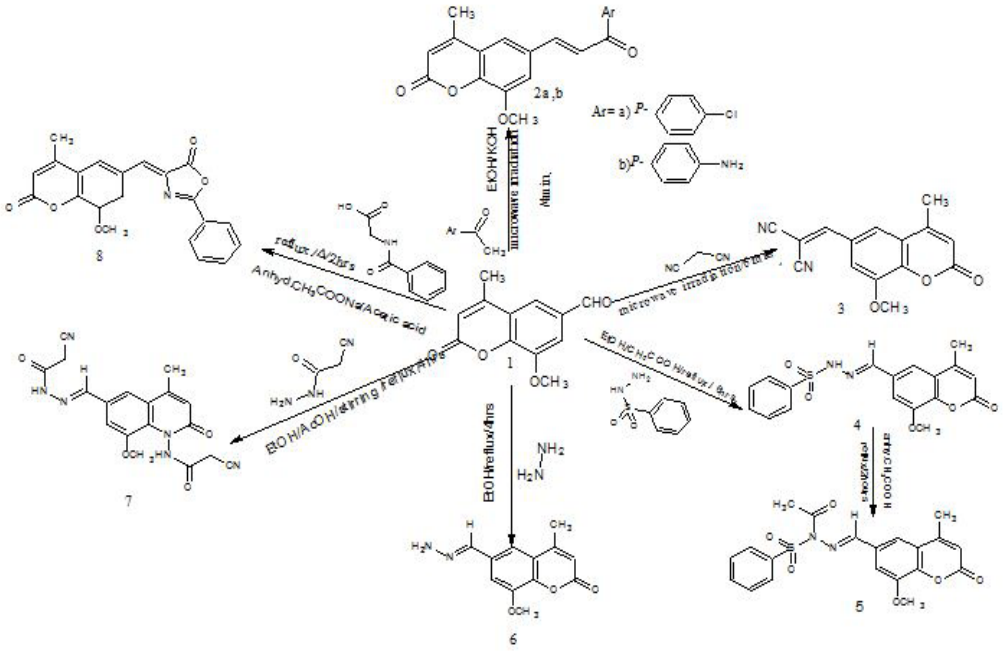
Coumarin agents (known as 1,2-benzopyrone), consisting of fused benzene and α-pyrone rings are present in significant amounts in plants and more than 1300 coumarins were identified from natural sources [1]. The synthesis of Coumarins and their derivatives has attracted considerable attention from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus [2]. Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine since ancient times. The antitumor activity of coumarin against human tumor cell lines was first noted by Weber et al. [3]. The selective tumor cell specific cytotoxicity of coumarins has been well documented by Riveiro et al. [4]. However, the use of coumarin in cancer chemotherapy was first established by the successful application of Warfarin sodium on V2 cancer cell, granulocytes, lymphocytes and macrophages in different animal models. Later, clinical trials demonstrated activity of coumarins in many different cancers, including prostate cancer, malignant melanoma and metastatic renal malignant melanoma and metastatic renal cell carcinoma [5].

Results and Discussion

The synthesis of the intermediate and target compounds was performed according to the reactions outlined in (Schemes 1-8) and the establishment of the structure of these compounds has been confirmed by spectroscopic data. The starting compound 1 was prepared following the reported literature procedure [6]. Compound **1** was reacted with acetophenone derivatives namely, 4-chloroacetophenone and 4-aminoacetophenone under microwave irradiation and solvent free conditions to afford 2a,b in excellent yield (Scheme 1) [7]. The structure of 2a was supported by IR spectrum which showed characteristic absorption band at 1723cm^{-1} attributable to δ-Lactone whereas, the IR spectrum of 2b showed absorption bands at 1727,3334 and 3299cm^{-1} attributable to δ-Lactone and NH_2 groups. The mass spectrum of 2a showed molecular ion peak at m/z 354.6(20% M^+). Similarly, compound 1 was reacted with active methylene compounds namely, malononitrile under solvent free and microwave irradiation conditions to yield the corresponding [(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl) methylidene] propanedinitrile 3 (Scheme1) [8,9]. The structure of compound 3 was elucidated on the basis of elemental analysis, spectral data and chemical transformation. The IR spectrum of 3 showed an absorption band at 2220cm^{-1} attributable to C≡N whereas the 1H-NMR spectrum revealed the presence of singlet

signals of three protons at δ 1.2 for CH_3, three protons at δ 3.8 for OCH_3 and one proton at δ 8.4 for $C(CN)_2$=CH.

Scheme: 1



Treatment of compound 1 with benzenesulfonohydrazide in ethanolic solution and few drops of acetic acid afforded the corresponding N'-[(E)-(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl) methylidene] benzene sulfonohydrazide 4 which was reacted with acetic anhydride to afford N'-[(E)-(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)methylidene]-N(phenylsulfonyl) acetohydrazide 5. The IR spectrum of 4 showed absorption bands at , 3349 , 1727, 1640 and 1088cm^{-1} attributable to NH, δ-Lactone, C=N and SO_2, functions. Its 1H NMR spectrum revealed singlet signals of one proton at δ 8.35 for NH and one proton at δ 7.59 for CH=N . The IR spectrum of 5 showed absorption band at 1763cm^{-1} for $COCH_3$ group and devoid the presence of NH. Its 1H-NMR revealed singlet signal of three protons at δ 2.53 for N-$COCH_3$ and disappearance of one Proton for NH group.

On the other hand, compound 1 was reacted with hydrazine derivatives namely; hydrazine hydrate and cyanoacetic hydrazide to afford the corresponding 6-[(E)-hydrazinylidenemethyl]-8-methoxy-4-methyl-2H-coumarin-2-one 6 and 2-cyano-N'-[(E)-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene] acetohydrazide **7**, consequently. The IR spectrum of **6** showed absorption bands at 3396.3, 3428, 1707, 1575 cm^{-1} indicated the presence of NH_2, δ-Lactone and C=N functions. Its ^{1}H-NMR revealed signals at δ 8.34, 8.887 (1H, s, CH=N-), (1H, s, NH) .The IR spectrum of **7** showed absorption bands at 1607.60cm^{-1}C=N, 1682.38 cm^{-1} CO-NH amide, 2183.33cm^{-1}CN and 3152.33 cm^{-1} NH . Its ^{1}H-NMR (DMSO-d6) spectrum showed signals at 1.410(3H,s,CH_3), 3.332, 3.524(4H, s, 2CH_2), 3.854(3H, s ,OCH_3), 6.251(1H, s, CH-coumarin), 7.919-7.550(2H, m, Ar-H), 8.362(1H, s, CH=N) and 8.889(1H, s, NH).

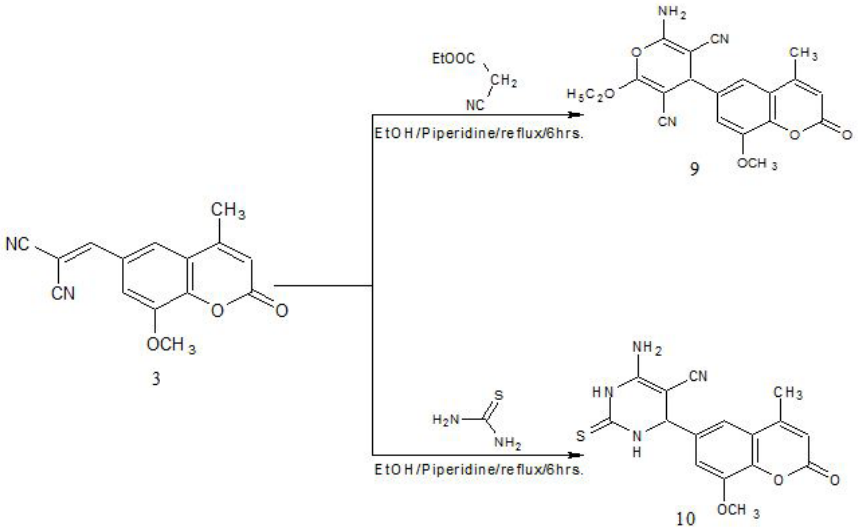
Also, 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde 1 was reacted with [(phenylcarbonyl) amino]acetic acid in the presence of acetic anhydride and sodium acetate under refluxing to give (5Z)-5-[(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)methyldene]-2-phenyl-1,3-oxazol-4(5*H*)-one 8 [10]. The IR spectrum of compound 8 indicated the presence of characteristic absorption bands at 1605.56 cm^{-1}C=N,1712.06 cm^{-1} δ-Lactone and 1759.44cm^{-1} C=O(Oxazole ring) .

Compound 3 was reacted with ethyl cyanoacetate in the presence of catalytic amount of piperidine under refluxing to afford 2-amino-6-ethoxy-4-(8-methoxy-4-methyl-2-oxo-2H-coumain-6-yl)-4H-pyran-3,5-dicarbonitrile 9 (Scheme 2) [11]. The IR spectrum of **9** showed absorption bands at 1646.7cm^{-1} C=C, 1720.6 cm^{-1} δ-Lactone, 2196.6 cm^{-1}CN and 3213.8 , 3336.9 $cm^{-1}NH_2$. The ^{1}H-NMR (DMSO-d6) spectrum showed signals at 1.224 (3H, s, CH_3), 1.646 (3H, t, CH_3CH_2) 4.250(2H, q, CH_2CH_3), 3.770 (3H, s, OCH_3), 6.409 (1H, s, CH-coumarin), 7.272-6.932 (3H, m, Ar-H) and 9.024(2H, s, NH_2) .

Similarly, Compound 3 was reacted with thiourea in the presence of of catalytic amount of piperidine under refluxing to gave (4E)-6-amino-4-[(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)methylidene]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-

carbonitrile 10 [12]. The IR spectrum of 10 showed absorption bands at 1203.3 cm^{-1}C=S, 1651.4 cm^{-1} C=N 1703.4cm^{-1} δ-Lactone, 2213.8 cm^{-1}CN, 3178.3cm^{-1}NH and 3243.8,3332.8$cm^{-1}NH_2$.

Scheme: 2a

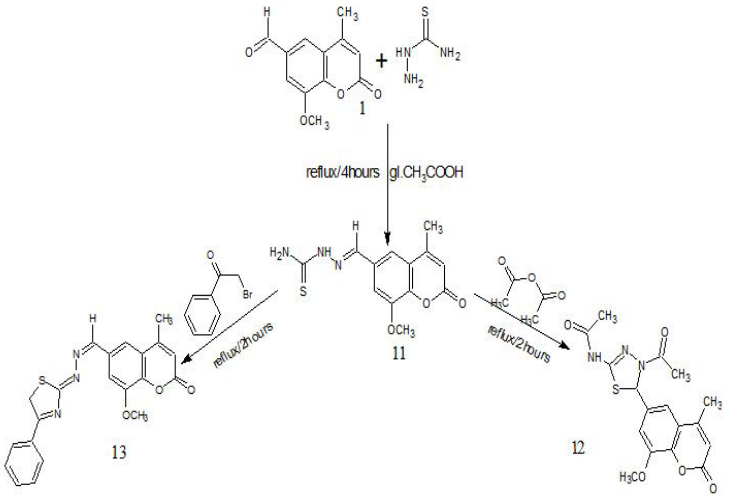


In addition, compound 1 was reacted with hydrazine carbothioamide in glacial acetic acid under refluxing to afford (2E)-2-[(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)methylidene] hydrazinecarbothioamide that formulated as 11 in excellent yield (Scheme 2) [13]. The IR spectrum of 11 showed absorption bands at 1285.35cm^{-1}C=S, 1645.2cm^{-1} C=N(imine),1696.92 cm^{-1} δ-Lactone, 2696.97cm^{-1} SH and 3371.0,3266.5cm^{-1} NH_2. ^{1}H-NMR (DMSO-d6) spectrum of 11 showed signals at 1.821(3H,s,CH_3),3.796(3 H,s,OCH_3),6.497(1H,s,CH-coumarin), 7.400-7.230(2H,m,Ar-H), 8.243(1H,s,CH=N), 9.070(1H, s, C-SH), 4.479(2H,s,NH_2) and 9.640(1H, s ,NH) . Its mass spectrum showed ion peak at m/z 291(0.01%).

Reaction of compound 11 with acetic anhydride under refluxing yielded N-[4-acetyl-5-(8-methoxy-4 methyl-2-oxo-2H-coumarin-6-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]acetamide 12 [13]. The IR spectrum of 12 showed absorption bands at 1614.4 cm^{-1}C=N, 1717.4 cm^{-1} δ-Lactone, 1763.0 cm^{-1} C=O (thiadiazole ring), and 3208.6cm^{-1}NH. Its 1H-NMR (DMSO-d6) spectrum showed signals at 1.245 (3H,s,CH_3), 1.348 (3H,s,CO-CH_3),1.909(3H, s, CO-CH_3), 3.757(3H, s ,OCH_3), 6.295(1H,s,CH-thiadiazol ring), 6.911(1H, s, CH-coumarin), 7.934-7.464(2H, m, Ar-H) and 8.372(1H, s ,NH) . Its mass spectrum showed ion peak at m/z m/z 375(0.01%) .

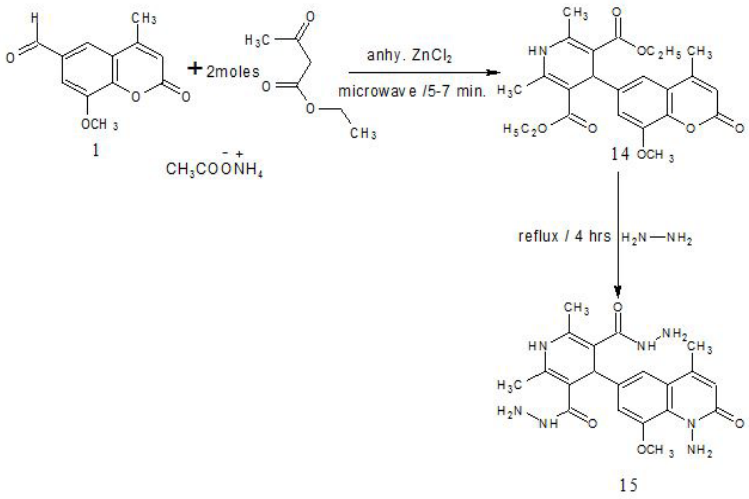
Also, treatment of compound 11 with 2-bromo-1-phenylethanone under refluxing lead to the formation of (2Z)-2-[(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene]-N-(2-oxo-2-phenylethyl) hydrazine carbothioamide 13 [14]. The IR spectrum of 13 showed absorption bands at 1549.01 cm^{-1}C=N and1710.23 cm^{-1} δ-Lactone. Its ^{1}H-NMR (DMSO-d6) spectrum showed signals at 1.055(3H, s ,CH_3), 2.498(2H, s, CH_2), 3.661(3H, s ,OCH_3), 6.355(1H, s, CH-coumarin), 7.945-6.678(7H, m, Ar-H) and 8.406 (1H, s, CH=N).

Scheme: 2b



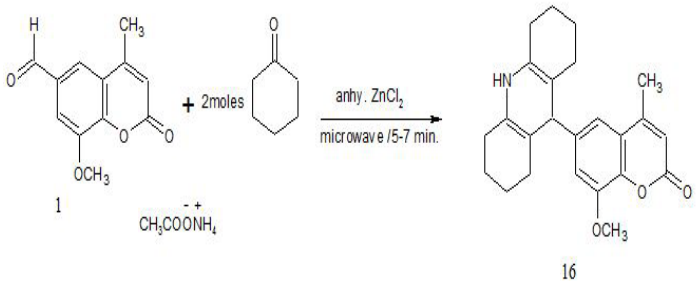
A mild and solvent-free conditions was used for synthesis of 1,4-dihydropyridine derivatives from ethyl acetoacetate, 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde 1 and ammonium acetate in the presence of zinc chloride as a catalyst under microwave irradiation conditions to give diethyl-4-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate 14 [15] . The IR spectrum of 14 showed absorption bands at 1647.7 cm^{-1}C=N, 1725.3 cm^{-1} δ-Lactone and 3269.5cm^{-1} NH . Its ^{1}H-NMR (DMSO-d6) spectrum showed signals at 1.060(3H, s ,CH_3), 1.318-1.209 (6H, m, 2XCH_3-pyridine ring), 4.258-3.980 (4H, m, CH_2) ,4.575(1H,S,CH-Pyridine) ,3.843(3H, s ,OCH_3), 5.908(1H, s, CH-coumarin), 7.386-6.540(2H, m, Ar-H) and 8.234 (1H, s, NH).

As consequently, The structure of 14 was confirmed by interaction with hydrazine hydrate in ethanolic solution under refluxing afforded the corresponding diethyl-4-(1-amino-8-methoxy-4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate 15. The IR spectrum of 15 showed absorption bands at 2260.8cm^{-1}CN, 2966.6-2936.6cm^{-1} CH_3 and 3299.8,3319.6cm^{-1} NH, NH_2 . Its mass spectrum showed ion peak at m/z 427.0(0.01%).



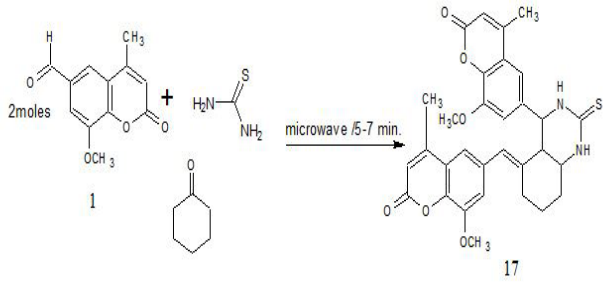
On the other hand, reaction of 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde 1 with cyclohexanone and ammonium acetate in 2:1:1M ratio in the presence of anhydrous zinc chloride under microwave irradiation conditions yielded 6-(1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)-8-methoxy-4-methyl-2H-coumarin-2-one 16 (Scheme 4) [15]. The IR spectrum of 16 showed absorption bands at 1702.4cm-1δ-Lactone and 3265.1cm-1 NH. Its mass spectrum showed ion peak at m/z 377(0.41%).

Scheme: 4

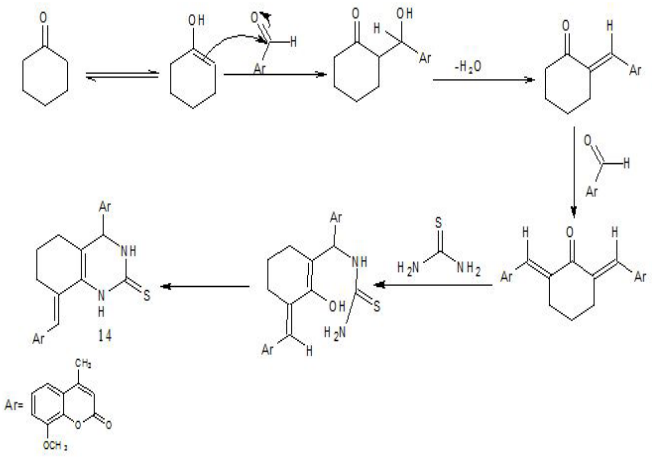


In the same manner, 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde 1 was reacted with thiourea and cyclohexanone in 2:1:1 M ratio under microwave irradiation conditions to give (E)-8-methoxy-6-(5-((8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylene)-2-thioxo-decahydroquinazolin-4-yl)-4-methyl coumarin-2-one17 (Scheme 5) [7]. The IR spectrum of 17 showed absorption bands at 1272.20cm-1C=S, 1597.94cm-1C=N, 1701.81cm-1 δ-Lactone and 3193.02cm-1NH. Its mass spectrum showed ion peak at m/z 554 which fragmented to give the base peak 202 (100%).

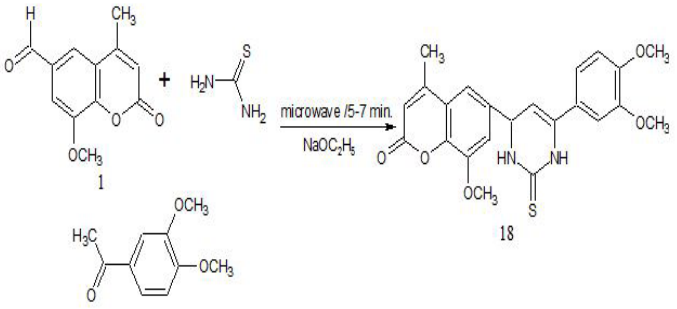
Scheme:5



The reaction takes place via the following mechanism [10];



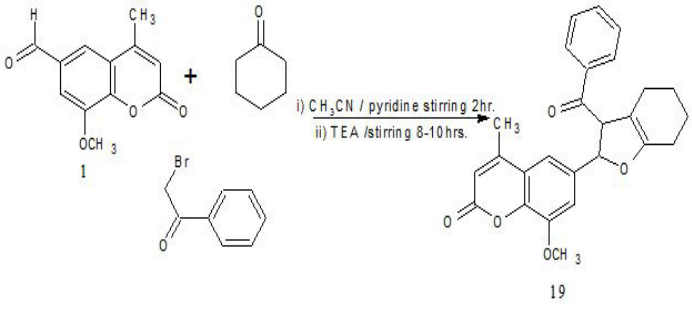
In the same manner, reaction of 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde 1 with 3,4-dimethoxyacetophenone and thiourea in the presence of sodium ethoxide under microwave irradiated gave 6-(6-(3,4-dimethoxyphenyl)-2-thioxo-2,3-dihydropyrimidin-4-yl)-8-methoxy-4-methyl-2*H*-coumarin-2-one 18 (Scheme 6) [16]. The infrared spectrum of 18 showed absorption bands at at 1199.22cm^{-1}C=S, 1617.60 cm^{-1}C=N, 1722.71cm^{-1} δ-Lactone and 3169.36 cm^{-1}NH . Its mass spectrum showed ion peak at m/z 496 which fragmented to give the base peak 75 (M^{+3}100%).



Scheme: 6

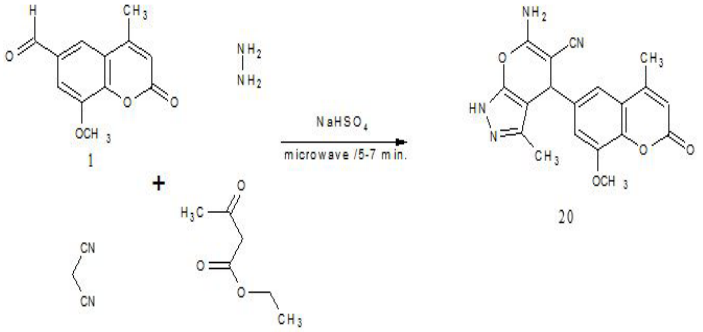
In addition, compound 1 was reacted with phenacyl bromide, cyclohexanone in acetonitrile and catalytic amount of pyridine to give 6-(3-benzoyl-2,3,4,5,6,7-hexahydrobenzofuran-2-yl)-8-methoxy-4-2H-coumarin-2-one 19 (Scheme 7) [17]. The infrared spectrum of 19 showed absorption bands at 1633.6 cm^{-1}C=O and 1704.0 cm^{-1} δ-Lactone. Its ^{1}H-NMR (DMSO-d6) spectrum showed signals at 1.18(3H, s ,CH_3), 2.499-2.444(2H, m, CH_2), 2.509-2.503 (2H, m, CH_2) ,3.707(3H, s ,OCH_3), 6.434(1H, s, CH-coumarin) 6.46(OCH-Furan), 3.32(COCH-Furan), 7.976-7.469 (7H, m, Ar-H).

Scheme: 7



Compound 1 was reacted with malononitrile and hydrazine hydrate under microwave irradiation in the presence of sodium bisulphate to give 6-amino-4-(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)-3-methyl-1,4-dihydropyrano [2,3-c] pyrazole-5-

carbonitrile 20 (Scheme 8) [18]. The infrared spectrum of 20 showed absorption bands at 1634.2 cm^{-1}C=N, 1715.5 cm^{-1} δ-Lactone, 2207.3 cm^{-1}CN and 3329.5,3204.9cm^{-1} NH_2. ^{1}H-NMR (DMSO-d6) spectrum which showed signals at 1.202(3H, s,CH_3), 2.074(3H, s, CH_3-pyrazol ring), 3.846(3H, s ,OCH_3), 5.494(1H,s,CH-Pyran), 6.365(1H, s, CH-coumarin), 7.951-6.509(2H, m, Ar-H), 4.180(2H, s,br, NH_2) and 12.953(1H,S,NH-Pyrazol).



Cytotoxicity Study

The anti-tumor activity of some newly synthesized coumarins against four cell lines: Human breast adenocarcinoma (MCF-7), colorectal carcinoma cells (HCT-116), hepatocellular carcinoma (Hep-G2) and prostate carcinoma (PC-3) has been evaluated by using MTT method [19, 20]. The cytotoxicity of the tested compounds (1, 5 and 15) at different concentrations against the four human cell lines. Compound (15) was the most potent against MCF-7 and HEP-G2 (IC50=12.9 and 11.6µg/ml, respectively), while compound (1) was most potent against HCT-116 (IC_{50}= 15.9 µg/ml) and compound (5) was most potent against PC-3 with IC_{50}= 26.7 µg/ml (Table 1).

Comp. Cell lines	IC50 (µg/ml)		
	Comp. (1)	Comp. (5)	Comp. (15)
MCF-7	40.4	20.4	12.9
HCT-116	15.9	21.5	48
HEP- G2	20.3	21.4	11.6
PC-3	40.5	26.7	>50

Table 1: The half maximal inhibitory concentration (IC_{50}) of the tested compounds against the different studied human cell lines.

The tested compounds down regulate significantly (p<0001) the expression of EGFR in both the MCF-7 and HEP-G2 cell lines, compared to their respective control cells (Figure 1).

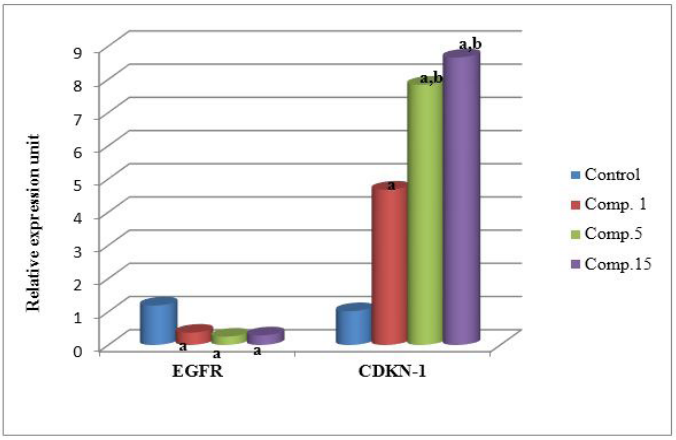


Figure 1: The mRNA level of EGFR and CDKN-1 in MCF-7 cell line.

As regard to CDKN 1, all tested compounds up regulate significantly (p<001) the expression of CDKN 1 in MCF-7 and HEP-G2, compared to their respective control cells (Figure 2).

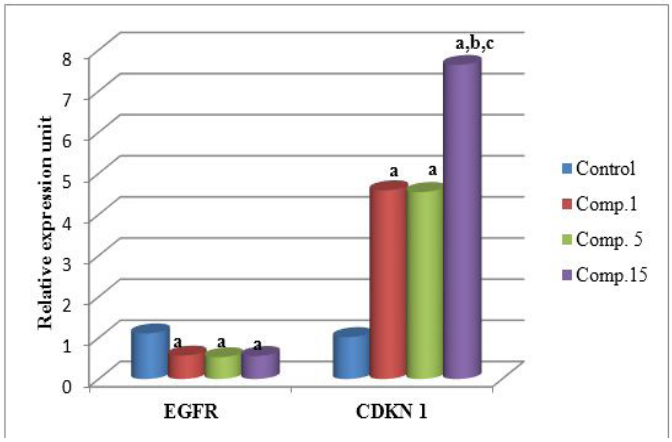


Figure 2: The mRNA level of EGFR and CDKN-1 in HEP-G2 cell line.

Multiple comparison analysis showed that compounds 5 and 15 increased significantly (p<0001) the expression of CDKN 1 in MCF-7 cell line, compared to compound 1 while in HEP-G2 cell line, compound 15 increased significantly (p<004 and 003) the expression of CDKN 1 as compared to compounds 1 and 5, respectively.

Experimental

All melting points are not corrected. The infrared spectra on KBr disks were carried on a Pye Unicam SP^3-200 spectrophotometer. The NMR Spectra were measured on a varian EM 60 and JE OL-

90 MHz Spectrometers with TMS as internal reference, chemical shifts were expressed in δ ppm. The mass spectra were determined on a FINNI-Gas 3300 mass spectrometer by direct inlet (Source temperature 90-300ºC, beam energy 70 ev).

General procedure

Synthesis of 8-methoxy-4-methyl-2-oxo-2H-coumarin-6-carbaldehyde (1)

A mixture of 4-hydroxy-3-methoxybenzaldehyde (0.01 mole; 1.09 gm), (0.01 mol) ethylaceto acetate in concentrated H_2SO_4 (25 ml) was heated with stirring on water bath for half an hours. The reaction product was poured onto ice/cold water, the solid that separated was crystallized from ethanol to give 1.

1: $C_{12}H_{10}O_4$ (M W218): dark green powder; 92% yield; m.p 168ºC. Anal calcd. C, 66.06;H, 4.59 . Found; C, 66.04;H, 4.57. FT-IR (cm-^{1}): 1616 cm^{-1} C=C, 1696 cm^{-1} C=O, 1703cm^{-1} δ-Lactone. δH=1.35 (3H,s,CH_3), 3.81(3H, s, OCH_3), 6.87(1H, s, CH-coumarin), 7.60-7.33 (3H, m, Ar-H) and 8.36 (1H, s, CHO).

Synthesis of 6-[(1E)-3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl]-4,8-dimethyl-2H-coumarin-2-one (2a) and 6-[(1E)-3-(4-aminophenyl)-3-oxoprop-1-en-1-yl]-4,8-dimethyl-2*H*-coumarin-2-one (2b)

To (0.01 mole; 2.18 gm) of compound **1** , (0.01mol; 1.54gm) 4-chloroacetophenone and /or (0.01mol; 1.54gm) 4-aminoacetophenone were heated under microwave irradiation for 4 minutes. The solid that separated was dried and crystallized from ethanol to give 2a,b.

2a: $C_{20}H_{15}ClO_4$ (MW 354.5); Dark brown crystals 80%yield; m.p.375^{0}C. Anal. Calcd for: C, 67.71; H, 4.23; Cl, 10.01: Found; C, 67.70; H, 4.21; Cl, 10.00. FT-IR (cm^{-1}): 669(C-Cl), 1723(δ-Lactone), 2983(CH-Coumarin). MS (m/z,%), 354.6(20%) [M$^+$].

2b: $C_{20}H_{17}NO_4$(M W335): blue powder with m.p. 325C^0 and yield 75% . Analy calc. for C, 71.64;H, 5.07; N, 4.18. Found; C, 71.62;H, 5.05;N, 4.16. IR (cm^{-1}): 1727 δ-Lactone, 2900CH-Coumarin and 3334-3299 NH_2.

Synthesis [(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl) methylidene] propanedinitrile 3

A mixture of 1 (0.01mol; 2.18gm) and (0.01mol; 0.66gm) propanedinitrile was heated under microwave irradiation and solvent free conditions for 6 minutes. The reaction product was recrystallized ethanol/petroleum ether (b.p.60-80˚C) (1:1) to give [(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl) methylidene] propanedinitrile 3.

3 as brown powder with m.p.260˚C and yield 70%. Analy. Calc. for $C_{15}H_{10}N_2O_3$ (266)C 67.67 ; H 3.67; N 10.53. Found: C 67.65; H_3.65; N 10.51. MS(m/z,%)391(5.90%)M$^+$. IR (cm^{-1}): at1605 cm^{-1} C=C, 1723 cm^{-1} δ-Lactone and 2220cm^{-1}CN. δH=1.23 (3H, s, CH_3) , 3.75 (3H,s,OCH_3) , 6.11(1H, s, CH-coumarin) , 8.39(1H, s, $C(CN)_2$=CH) ,7.96-7.11 (2H, m, Ar-H) .

Synthesis of N'-[(E)-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl) methylidene] benzene sulfonohydrazide 4.

A mixture of 1 (0.01mol; 2.18gm) and (0.01mol;1.72gm) benzene sulfonohydrazide in ethanolic solution (25ml) and drops of acetic acid was refluxed for 6 hrs. The result solution was poured into crushed ice/water . The solid that separated was filtered off, dried and recrystallized from ethanol/water to give N'-[(E)-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl) methylidene] benzene sulfonohydrazide 4**.**

4 as gray powder with m.p 195ºC and yield 70% . Anal. Calc. for $C_{18}H_{16}N_2O_5S$ (372): C 58.06; H 4.30; N 7.53 ; S 8.61. Found: C 58.04; H 4.28; N 7.51 ; S 8.60. IR(cm^{-1}) 1088.82cm^{-1}SO_2, 1570 cm^{-1}C=C,1640 cm^{-1}C=N, 1727 cm^{-1} δ-Lactone and 2920-3054 cm^{-1} CH(aliphatic-aromatic), 3349 cm^{-1} NH .δ H=1.11(3H, s ,CH_3), 8.35(1H, s ,NH), 3.66(3H, s ,OCH_3), 6.21(1H, s, CH-coumarin), 7.59-6.65 (7H, m, Ar-H) and 7.59(1H,s,CH=N).

Synthesis of (5Z)-5-[(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl) methylidene]-2-phenyl-1,3-oxazol-4(5H)-one 5

A mixture of 1 (0.01 mol; 2.18gm) and [(phenylcarbonyl) amino]acetic acid (0.01mol; 1.79gm) in the presence of acetic anhydride and sodium acetate was heated under refluxing for 2 hours. The product obtained was poured into crushed ice, filtered off, dried then, recrystallized from ethanol/petroleum ether (b.p.60-80˚C) (1:1) to give 5

5 as brown powder with m.p. > 300ºC(420ºC) and yield 60%. Anal. Calc. for $C_{21}H_{17}NO_5$ (363): C 69.41; H 4.72 ; N 3.85 .Found: C 69.40; H 4.70;N 3.82. MS m/z 361(2.08%) and base peak at m/z 64(100%) . IR(cm^{-1}): 1605 cm^{-1}C=N, 1712 cm^{-1} δ-Lactone and1759 cm^{-1} C=O(oxazole ring) .

Synthesis of 6-[(E)-hydrazinylidenemethyl]-8-methoxy-4-methyl-2*H*-coumarin-2-one 6

(0.01mol; 2.18gm) of 1 (0.01mol) of hydrazine hydrate in ethanolic solution (25ml) were heated under refluxing and stirring for 4 hrs. The precipitate was washed well, dried and recrystallised from ethanol to give 6.

6 as Pale yellow crystals with m.p.235^{0}C and yield 85%. Anal. Calc. for $C_{12}H_{12}N_2O_3$ (232): C 62.07; H 5.17; N 12.07. Found: C 62.05; H 5.15; N 12.05. IR(cm^{-1}): 1574 cm^{-1} C=N,1707 cm^{-1} δ-Lactone and 3396 ,3428 cm^{-1} NH_2 . δH=1.18 (3H, s ,CH_3), 3.79 (3H, s ,OCH_3), 8.34(1H, s, CH=N-), 6.29(1H, s, CH-coumarin), 7.40-6.78(7H, m, Ar-H) and 8.89(1H, s, NH).

Synthesis of 2-cyano-N'-[(E)-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene]aceto hydrazide 7

(0.01mol; 2.18gm) of 1, (0.02mol) 2-cyanoacetohydrazide in ethanolic solution (25ml) and in the presence of drops of

acetic acid were heated under refluxing and stirring for 4 hrs. The precipitate was washed well, dried and recrystallised from ethanol to give **7** .

7 as Dark brown crystals with m.p. 218^{0}C and 70% yield, anal calc for $C_{15}H_{13}N_3O_4$ (299): C 60.20; H 4.35 ; N 14.05 . Found: C 60.20; H 4.32; N 14.03 . IR(cm^{-1}): 1607cm^{-1}C=N, 1682 cm^{-1}CO-NHamide, 2183cm^{-1}CN and 3152 cm^{-1} NH. δH=1.41(3H, s ,CH_3), 3.33, 3.52(4H, s, 2CH_2), 3.85(3H, s ,OCH_3), 6.25(1H, s, CH-coumarin), 7.92-7.55(2H, m, Ar-H), 8.36(1H, s, CH=N) and 8.89(1H, s, NH).

Synthesis of N'-[(E)-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene]-N-(phenylsulfonyl) aceto-hydrazide 8

A mixture of 4 (0.01mol; 3.72gm), (0.01mol) acetic anhydride was heated under refluxing for 2 hour. After cooling, the reaction mixture poured slowly onto crushed ice/H_2O. The solid that separated was filtered off, washed well with water, dried and recrystallized from ethanol to give 8.

8 as dark brown powder in 65 % yield and m.p. 170ºC. anal calc for $C_{20}H_{18}N_2O_6S$ (414): C57.97 ;H 4.35; N 6.76 ; S7.73; Found: C 57.95; H 4.33; N 6.75; S 7.71. IR (cm^{-1}): 1074cm^{-1} SO_2,1610 cm^{-1}C=N, 1725cm^{-1} δ-Lactone, 1763cm^{-1} CH_3C=O and devoid of NH. δ H= 1.19(3H, s ,CH_3), 2.53(3H, s ,N-C=O(CH_3) , 3.68(3H, s, OCH_3), 6.14(1H, s, CH-coumarin), 7.64-6.84(7H, m, Ar-H) and 7.89(1H, s, -N=CH-aldimine).

Synthesis of 2-amino-6-ethoxy-4-(8-methoxy-4-methyl-2-oxo-2H-coumain-6-yl)-4H-pyran-3,5-dicarbonitrile 9

A mixture of 3 (0.01 mol; 2.66 gm), ethyl cyanoacetate (1.13 mol) and catalytic amount of piperidine in ethanolic solution (50ml) was heated under refluxing for 6 hrs. The excess solvent was then removed by distillation and the reaction residue poured onto crushed ice/water. The separated solid was filtered off, dried and recrystallized from acetone/water (1:1) to give 9

9 as a violet powder with m.p.> 380˚C and yield 90%. anal. Calc. for $C_{20}H_{17}N_3O_5$ (379): C 63.32; H 4.52; N 11.08; Found: C 63.30; H 4.50; N 11.06. IR (cm^{-1}): at 1720 cm^{-1} δ-Lactone, 2196 cm^{-1} CN, 2851-2955 cm^{-1}, 3213 cm^{-1} and 3336 cm^{-1} NH_2. δH=1.22(3H, s, CH_3), 1.65(3H, t, CH_3CH_2) 4.25(2H, q, CH_2 CH_3), 3.77(3H, s, OCH_3), 6.41(1H, s, CH-coumarin), 7.27-6.93 (3H, m, Ar-H) and 9.02(2H, s, NH_2) .

Synthesis of (4E)-6-amino-4-[(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile 10

(0.01 mol; 2.66 gm) of 3 reacted with (0.01mol; 0.76gm) of thiourea in ethanolic solution (25ml) was refluxed for 6 hrs. The solid that separated after concentration was filtered off, dried and recrystallized from ethanol/water to give 10 .

10 as a brown powder with m.p 350ºC and yield 75 %. Anal. Calc for $C_{16}H_{14}N_4O_3S$ (342): C, 56.13; H, 4.12; N, 16.36; S, 9.37; Found: C, 56.11; H, 4.10; N, 16.34; S, 9.35. IR(cm^{-1}): 1203 cm^{-1}C=S, 1651 cm^{-1}C=N, 1703cm^{-1} δ-Lactone, 2213 cm^{-1}CN, 3178cm^{-1} NH and 3243, 3332cm^{-1} NH_2. δH=1.15(3H, s ,CH_3), 3.72(3H, s,OCH_3), 4.60(2H, s ,NH_2), 6.24(1H, s, CH-coumarin), 7.83 - 6.82 (2H, m, Ar-H), 8.30, 7.95(2H, s,2NH).

Synthesis of (2E)-2-[(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylidene] hydrazine carbothioamide 11.

A mixture of 1 (0.01 mol; 2.18gm) and hydrazine carbothioamide (0.01mol; 0.91gm) in glacial acetic acid was refluxed for 3 hours. After cooling, the solid that separated by filteration, washed well with water, dried and recrystallized from acetone/ethanol (1:1) to give 11.

11 as a yellow crystals with m.p. 202ºC and yield 90 %. anal calc for $C_{13}H_{13}N_3O_3S$ (291):C, 53.61; H, 4.47; N, 14.43; S, 11.01; Found: C, 53.60; H, 4.45; N, 14.41; S, 11.00, MS m/z 291(0.01%) and base peak at m/z 91.06 (100%). IR (cm^{-1}): 1285cm^{-1}C=S, 1619cm^{-1}C=C, 1645cm^{-1} C=N (imine),1696 cm^{-1} δ-Lactone, 2696cm^{-1} SH,3025-2970 cm^{-1} CH (aliphatic-aromatic), 3371-3266cm-1 NH_2. δH=1.82(3H, s ,CH_3), 3.80(3H, s ,OCH_3), 6.50(1H, s, CH-coumarin), 7.40-7.23(2H, m, Ar-H), 8.24(1H, s, CH=N), 9.10(1H, s, C-SH), 4.48(2H,s,NH_2) and 9.64(1H, s ,NH) .

Synthesis of N-[4-acetyl-5-(8-methoxy-4-methyl-2-oxo-2*H*-coumarin-6-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]acetamide 12

Acetic anhydride (40ml) was added to (0.01 mol; 2.91gm) of 10 and heated under refluxing for 4 hours. After cooling, the solid product was filtered off, washed well with water and recrystallized from ethanol/water (1:1) to give 12.

12 as violet crystals with m.p. 320ºC and yield 70 % ; anal calc for $C_{17}H_{17}N_3O_5S$ (375): C, 54.39; H, 4.53 ; N, 11.19; S, 8.53; Found: C, 54.37; H, 4.51; N, 11.17; S, 8.51. MS m/z 375(0.01%) and its base peak at m/z 44(100%). IR(cm^{-1}) 1614 cm^{-1}C=N, 1717 cm^{-1} δ-Lactone , 1763cm^{-1} C=O (thiadiazole ring) and 3208 cm^{-1}NH. δH=1.25(3H, s ,CH_3), 1.35 (3H, s, CO-CH_3),1.91(3H, s, CO-CH_3), 3.76(3H, s ,OCH_3), 6.30(1H, s ,CH-thiadiazol ring), 6.91(1H, s, CH-coumarin), 7.93-7.46(2H, m, Ar-H) and 8.37(1H, s ,NH) .

Synthesis of (2Z)-2-[(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)-methylidene]-N-(2-oxo-2-phenylethyl) hydrazine carbothioamide 13

To ethanolic solution of 10, (0.01mol;1.99 gm) 2-bromo-1-phenylethanone was added. The reaction mixture was heated under refluxing for 8 hrs. The solid that separated after concentration and cooling was filtered off and recrystallized from ethanol to give 13 .

13 as brown crystals with m.p. 298ºC and yield 80%**,** anal. calc. $C_{21}H_{17}N_3O_3S$ (391); C, 64.43; H, 4.38; N, 10.73; S, 8.19;

Found: C, 64.41; H, 4.36; N, 10.71; S, 8.17. IR(cm-^{1}) 1549 cm-^{1}C=N, 1710 cm^{-1} δ-Lactone. δH=1.06(3H, s ,CH_3), 2.50(2H, s, CH_2), 3.66(3H, s ,OCH_3), 6.36(1H, s, CH-coumarin), 7.95-6.68(7H, m, Ar-H) and 8.41 (1H, s, CH=N) .

Synthesisofdiethyl4-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate 14

(0.01mol; 2.18) gm1 reacted with (0.02mol; 2.66gm) ethyl acetoacetate and (0.01mol; 0.77gm) ammonium acetate under microwave irradiation for 7 minutes in the presence of anhydrous zinc chloride as a catalyst. The solid that separated was washed well with water, filtered off, dried and recrystallized from ethanol/acetone/water (1:1:1) to give 14

14 as reddish brown powder with m.p >300ºC and yield 80% .Anal. Calc.: $C_{24}H_{27}NO_7$ (414); C 65.29; H 6.16; N 3.17. Found: C 65.27; H 6.14; N 3.15.IR (cm^{-1}); 1647 cm^{-1}C=N, 1725 cm^{-1} δ-Lactone and 3269cm^{-1} NH. δH=1.06(3H, s ,CH_3), 1.32-1.21(6H, m, 2XCH_3-pyridine ring), 4.26-3.98 (4H, m, CH_2) ,4.58(1H,S,CH-Pyridine) ,3.85(3H, s ,OCH_3), 5.91(1H, s, CH-coumarin), 7.39-6.54(2H, m, Ar-H) and 8.23 (1H, s, NH).

Synthaesis of diethyl 4-(1-amino-8-methoxy-4-methyl-2-oxo-1,2-dihydroquinolin-6-*yl*)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate 15.

A mixture of (0.01 mol; 4.41 gm) of 13 and (0.01mol) hydrazine hydrate in ethanolic solution was reacted under refluxing for 4 hours . The reaction mixture was allowed to cool .The solid that separated was filtered off, dried and recrystallized from ethanol/water (1:1) to give 15.

15 as pale brown crystals with m.p. 270ºC and yield 85 %. Anal. Calc.: $C_{20}H_{25}N_7O_4$(427); C 56.20; H 5.89; N 22.94. Found: C 56.18 ; H 5.87; N 22.92. MS m/z 427.0(0.01%) and base peak at m/z 44(100%). IR (cm^{-1}); 2260cm^{-1}CN, and 3299, 3319cm^{-1} NH, NH_2.

Synthesis of (E)-8-methoxy-6-(5-((8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)methylene)-2-thioxo-2,4a,5,6,7,8-hexahydroquinazolin-4-yl)-4-methyl-2H-coumarin-2-one 16.

A mixture of (0.02mol; 4.36gm) of 1 , (0.01mol; 0.98gm) cyclohexanone and (0.01mol; 0.76gm) thiourea was reacted under microwave irradiation for 7minutes. The solid that separated was washed well with water, filtered off, dried and recrystallized from ethanol/water (1:1) to give 16.

16 as dark brown powder with m.p. > 300ºC and yield 80% . Anal. Calc.: $C_{31}H_{30}N_2O_6$ (558); C 66.65; H 5.41 ; N 5.01; S 5.74 . Found: C 66.63; H 5.40; N 5.00; S 5.72. MS m/z 554 which fragmented to give the base peak 202 (M^{+2}100%). IR (cm^{-1}); at 1272cm^{-1} C=S, 1597cm^{-1}C=N,1701cm^{-1} δ-Lactone and 3193cm^{-1}NH .

Synthesis of 6-(1,2,3,4,5,6,7,8,9,10-decahydroacridin-9-yl)-8-methoxy-4-methyl-2H-coumarin-2-one 17

(0.01mol; 2.18gm) of 1 reacted with (0.02mol; 2.66gm) cyclohexanone and (0.01mol; 0.77gm) ammonium acetate under microwave irradiation for 7 minutes in the presence of anhydrous zinc chloride as a catalyst. The solid that separated was washed well with water, filtered off, dried and recrystallized from ethanol/acetone/water (1:1:1) to give 17

17 as dark gray powder with m.p. > 300ºC and yield 80% . Anal. Calc. : $C_{24}H_{27}NO_3$(377); C76.36; H 7.21; N 3.71. Found: C76.35; H 7.20; N 3.70.MS m/z 377(0.41%) and its base peak at m/z 80(100%). IR (cm^{-1});1702cm^{-1}δ-Lactone, and 3265cm^{-1} NH.

Synthesis of 6-(6-(3,4-dimethoxyphenyl)-2-thioxo-2,3,4,5-tetrahydropyrimidin-4-yl)-8-methoxy-4-methyl-2-oxoquinoline-1(2H)-carbothioamide 18.

A mixture of (0.01mol; 2.18gm) of 1,(0.01mol; 1.80gm) 3,4-dimethoxyacetophenone and (0.01mol; 0.76gm) thiourea was reacted in the presence of sodium ethoxide solution under microwave irradiation for 7minutes. The solid that separated was washed well with water, filtered off, dried and recrystallized from ethanol/petroleum ether(b.p.60-80˚C) (1:1) to give 18

18 as brown powder with m.p. > 300ºC and yield 80%. Anal. Calc.: $C_{23}H_{20}N_2O_5S$ (436); C63.29; H 4.62; N 6.40; S 7.35. Found: C63.27; H 4.60; N 6.40; S 7.33 MS m/z 496 which fragmented to give the base peak 75 (M^{+3}100%). IR (cm^{-1});1702cm^{-1}δ-Lactone, and 3265 cm^{-1} NH .

Synthesis of 6-(3-benzoyl-2,3,4,5,6,7-hexahydrobenzofuran-2-yl)-8-methoxy-4-2H-coumarin-2-one 19.

A mixture of (0.01mol; 2.18gm) of 1, (0.01mol; 0.98gm) cyclohexanone and (0.01mol; 1.99gm) 2-bromo-1-phenylethanone in the presence of acetonitrile solution and catalytic amount of pyridine was reacted under stirring for 2 hours at rt. Triethylamine (1ml) was added with continuous stirring. The product obtained was poured into crushed ice. The solid that separated was filtered off, dried and recrystallized from ethanol /water (1:1) to give 19.

19 as reddish brown powder with m.p. 320ºC and yield 80%. Anal. Calc.; $C_{26}H_{24}O_5$ (416); C 74.98; H 5.81. Found C74.96; H 5.80 . IR (cm-^{1}) 1633 cm^{-1}C=O and 1704 cm^{-1} δ-Lactone. δ H= 1.18(3H, s ,CH_3), 2.50-2.44(2H, m, CH_2), 2.51-2.50 (2H, m, CH_2) ,3.71(3H, s ,OCH_3), 6.43(1H, s, CH-coumarin) 6.46(OCH-Furan),3.32(COCH-Furan),7.98-7.47 (7H, m, Ar-H) .

Synthesis of 6-amino-4-(8-methoxy-4-methyl-2-oxo-2H-coumarin-6-yl)-3-methyl-1,4-dihydro- pyrano[2,3-c]pyrazole-5-carbonitrile 20

A mixture of (0.01mol; 2.18gm) of 1 (0.01mol; 1.33gm) ethyl acetoacetate, (0.01mol; 0.66 gm) malononitrile and (0.01mol) hydrazine hydrate was reacted under microwave irradiation for 7 minutes in the presence of sodium bisulphite. The solid that separated

was washed well with water, filtered off, dried and recrystallized from ethanol to give 20.

20 as reddish brown powder with m.p 380ºC and yield 85%. Anal. Calc. $C_{19}H_{16}N_4O_4$ (364); C62.63; H 4.43; N 15.38. Found; C 62.61; H 4.41; N 15.36. IR (cm^{-1}) ; 1634 cm^{-1}C=N, 1715 cm^{-1} δ-Lactone, 2207 cm^{-1} CN and 3329,3204cm^{-1} NH_2. ^{1}H-NMR (DMSO-d6) spectrum which showed signals at 1.20(3H, s ,CH_3), 2.07(3H, s, CH_3-pyrazol ring), 3.85(3H, s ,OCH_3), 5.49(1H,s,CH-Pyran), 6.37(1H, s, CH-coumarin), 7.95-6.51(2H, m, Ar-H) and 4.18(2H, s,br, NH_2) and 12.95(1H,S,NH-Pyrazol). 21

5.2. Cytotoxicity Assay

The cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay . Briefly, the cells were seeded in 96-well microtitre plate (Falcon, NJ, USA) at a cell concentration of 1x 10^4 cells per well in 100µ l of growth medium. Fresh medium containing different concentrations of the tested compounds was added after 24 hours of seeding. Serial two fold dilutions of the metabolites were added confluent cell monolayer. The micro titer plates (polystyrene sterile tissue culture plates) were incubated at 37°C in a humidified incubator with 5 % CO_2 for a period of 48 hours. Three wells were used for each concentration of the tested compounds. Control cells were incubated without the tested compounds and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 hours at 37°C, various concentrations of the tested compounds were added, and the incubation was continued for 48 hours. After the end of the incubation period, crystal violet solution (1%) was added to each well for 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid was then added to all wells and mixed thoroughly; the plates were read on ELISA reader, using a test wavelength of 490 nm. Treated samples were compared with the control. All experiments were carried out in triplicate. The percent cytotoxicity was calculated by the formula: percent cytotoxicity (cell death) = [1-(absorbance of experimental wells/absorbance of control wells)] × 100%.

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