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Design, Synthesis and Biological Evaluation of some Arylidenenitrile Derivatives Anticancer Agent

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Abstract: A series of arylidenenalononitrile esters**1-9** and arylidenenitriles**10-16**supported with some functionalities reported to contribute to significant chemotherapeutic potential were synthesized and evaluated for their cytotoxic activity. nine compounds exhibited cytotoxic potential against a panel of three human tumor cell lines. Compounds **11**, **12,14** and **15**proved to be the most active agents with a broad spectrum of cytotoxic activity. Analogs **11**and **14**were considered as the most active cytotoxic agent, being about two times more active than doxorubicin against the colon HT29 carcinoma cell line.

Keywords: Synthesis,Arylidenenitrile esters, ,Arylidenemalononitriles, Cytotoxicity.

1.Introduction

Cancer has been known as one of the most impressive clinical problems in both developing and developed countries. In spite of improved diagnostic techniques and advances in prevention and chemotherapeutic management of cancer, the disease still afflicts millions of peoples in the world [1]. Cancer cells are defined by uncontrolled replications associated with self-sufficiency in growth signals, hyposensitivity to anti-growth signals, ongoing angiogenesis, metastasis, and evasion of apoptosis [2]. Anti-cancer agents cannot recognize cancer cells from normal cells, as a matter of fact, these agents usually act on metabolically active or rapidly proliferating cells [3]. Thus, there has been increscent interest in the field of cancer chemotherapy by discovery and development of novel agents with high efficacy, low toxicity, and minimum side effects.

During recent years, several researchers developed different chalcone-like compounds with anticancer activity through the introduction of heterocyclic scaffolds [4,5]. The chemical

structure of chalcone is characterized by two aromatic rings connected by a three carbon, α,βunsaturated carbonyl system (1,3-diphenyl-2-propen-1-one) [6-8]. The highly significant advantage of chalcone derivatives as cytotoxic agents is the low propensity to interact with DNA; which omits the risk of mutagenesity as the common side effect of current chemotherapeutic agents [9].

Previously, Perjési et al. have reported cytotoxicity of 3-benzylidene-4-chromanones as rigid analogs of chalcones[10]. Recently, high-throughput screening of drug libraries results in the identification of SJ-172550 that exhibited p53-dependent cytotoxic activity against cancer cell lines [11]. Structurally, SJ-172550 is characterized by having α,βunsaturated carbonyl system attached to the 2-(2-chloro-6-ethoxyphenoxy)acetic acid methyl ester. Accordingly, in continuation of our research program to find novel anti-cancer agents [12-16] and considering the diverse biological activities of rigid chalcones[17], we have synthesized a series of 3-benzylidene-4-chromanones bearing 2-(2-chloro-6-alkoxyphenoxy) acetic acid esters. The related analogs of 3-benzylidene-4-chromanones were also prepared for more studying of structure-activity relationships.

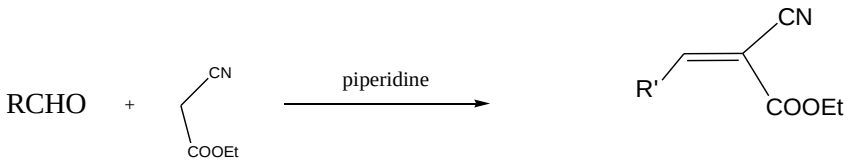
On the other hand,reagentsmalononitrile and ethylcyanoacetate are methylene active compounds largely used in the Knoevenagel condensation, an important C–C bond forming reaction which has been extensively studied[18] .The ylidenenitriles thus obtained have found increasing applications in industry, agriculture, medicine and biological science.[2] They are important intermediates for the synthesis of various organic compounds, mainly by cyclization reactions.[19,20]Indeed, different kinds of nitrogen and oxygen-ontaining heterocycles were obtained *e.g.* pyridines,[21b,f,i]pyrans,[21c,d,f,i] pyrimidines,[21i]pyranopyrimidines,[21d,g]pyranopyrazoles[21d,f] and phthalazines[21e]. Moreover, benzylidenemalononitrileswere reported to be effective anti-fouling agents, fungicides and insecticides. The chemical properties of benzylidene- malononitriles and their effects on, and interactions with, living organisms were extensively reviewed by Jones,[22]due to their use as cytotoxic agents against tumours or as riot control agents. Hydroxylated benzylidenemalononitrileswere described as protein tyrosine kinase inhibitors with antiproliferative activity.[23]

2. Results and discussion

2.1 Chemistry

Condensation of ethyl cyanoacetate and malononitrile and with some aromatic and heterocyclic aldehydes in presence of catalytic amount of piperidine afforded the corresponding 2-cyano-3-subistituted acylic acid ethyl ester **1-9** andarylidenemalononitrile derivatives **10-16** respectivelyin excellent yields (*Scheme 1& 2*). The IR spectra of theabove compounds**1-16** revealed absorption bands at 2224-2235 cm^{-1} attributed to the CN group. In addion the arylidine ester derivatives **1-9** exhibited another strong absorption at 1708-1720 cm^{-1}characteristic for the ester carbonyl band. Their structure was further confirmed from their ^{1}HNMR which exhibited beside the aromatic protons a singlet of one proton intensity at δ 7.80-8.21 for the olifinic proton. In addition compounds **1-9** showed the CH_3 and CH_2 of the ester group as triplet and quartet at 1.28-1.29 and 4.22-4.40 respectively. The structures were furthersupported from their^{13}C NMR spectral data which showed the expected number of aliphatic and aromatic carbons (experimental section).

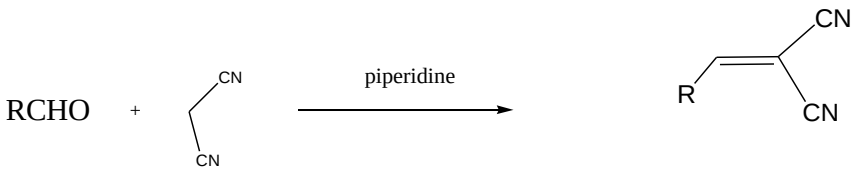
Scheme 1.



1-9

Compound	R	Compound	R
1	**4-$CH_3C_6H_4$**	**6**	4, 3-$(OCH_2O)C_6H_3$
2	**4-$CH_3OC_6H_4$**	**7**	**C_6H_5CH=CH**
3	**4-$(CH_3)_2NC_6H_4$**	**8**	**2-Theinyl**
4	**4-BrC_6H_4**	**9**	**2-Furyl**
5	**4-OH-3-CH_3OC_6H**		

Scheme 2



10-16

Compound	R	Compound	R
10	**4-$CH_3C_6H_4$**	**14**	**4-OH-3-$CH_3OC_6H_3$**
11	**4-$CH_3OC_6H_4$**	**15**	**4, 3-$(OCH_2O)C_6H_3$**
12	**4-$(CH_3)_2NC_6H_4$**	**16**	**C_6H_5CH=CH**
13	**4-BrC_6H_4**		

2.2 In VitroMTT Cytotoxicity Assay.

All the synthesized compounds were evaluated for their *in vitro* cytotoxic effect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] method[24,25]against a panel of three human tumor cell lines, namely, colon carcinoma HT29, hepatocellular carcinoma HePG2, and Caucasian breast adenocarcinoma MCF7. The results are presented in Table 1 as LC50 (μM) which is the lethal concentration of the compound that causes death of 50% of the cells in 24 h. The obtained data revealed that the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards nine of the tested compounds, namely,**2,3,4,8,11,12,13,14,** and **15**whereas the rest compounds were either marginally active or even totally inactive. Regarding the activity against the human colon carcinoma HT29, this cell line proved to be very sensitive to all the six active compounds. In particular, it revealed distinctive sensitivity towards compounds **11,14**and **15**(LC50 26.4, 28.2 and 29.5μM, resp.) even higher than doxorubicin (LC50 40.0 μM), the reference standard cytotoxic agent utilized in this assay. Meanwhile, compounds **12**(LC50 45.4 μM) were nearly equipotent to doxorubicin (LC50 40.0 μM), whereas compounds **13,3c**and **8**(LC50 64.2,74.5 and 84.6 μM, resp.) showed moderate cytotoxic potential against the same cell line. Shifting to the hepatocellular carcinoma HepG2, this cell line showed mild to weak sensitivity towards four of the tested analogs with LC50 range 58.5-110.6μM, when compared to doxorubicin (LC50 3.0 μM). Among these, the highest activity was displayed by compounds **11**and **14**(LC50 58.5 and 54.2 μM, resp.). On the other hand, the human breast

cancer MCF 7 emerged as the least sensitive among the cell lines tested as its growth was affected by the presence of only four test compounds. However, a remarkable growth inhibition potential was shown by analogs **11,14** and **15**as evidenced from their LC50 values (LC50 10.2, 12.4 and 13.5 μM, resp.),which represents about 40–60%of the activity of doxorubicin (LC50 4.0 μM). Further interpretation of the results revealed that compounds **11,12,14,15** and **8**showed considerable broad spectrum cytotoxic activity against the three tested human tumor cell lines. In particular, compounds **11,14** and **15**proved to be the most active members in this study with special effectiveness against both the colon carcinoma HT29 (almost twice as active as doxorubicin; LC50 26.4, 28.2 and 29.0 versus 40 μM, resp.) and human breast cancer MCF 7 (about 40– 60% of the activity of doxorubicin; LC50 10.2, 12.4 and 13.5 versus 4.0 μM, resp.).

A close examination of the structures of the active compounds showed that the presence of two cyano groups on the olefinic carbon seemed to influence the cytotoxic activity. In this context, the arylidenemalononitrilederivatives (**11-15**) were in favor of better cytotoxic activity, when compared with their ylidenenitrile ester congeners (**2,3,4** and **8**), as revealed from their LC50 values in Table 1.

Table 1. Cytotoxic effects LC_{50} (μM)[a]of the active compounds on some human tumor cell lines using the MTT assay.

Compound no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
11	**26.4**	**58.5**	**10.2**
12	**45.4**	**98.3**	**-[b]**
13	**59.1**	**-**	**-**
14	**28.2**	**54.2**	**12.4**
15	**29.0**	**52.8**	**13.5**
2	**50.4**	**88.6**	**-**
3	**74.5**	**-**	**-**
4	**64.8**	**105.6**	**-**
8	**84.6**	**110.6**	**86.3**
Doxorubicin [c]	**40.0**	**3.0**	**4.0**

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24h (μM). [b]Totally inactive against this cell line. [c] positive control cytotoxic agent.

3. Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

General procedure for the synthesis of the arylidene derivatives 1 -16

A mixture of the appropriate aldehyde (10 mmol), malononitrile or ethyl cyanoacetate (10 mmol) and catalytic amount of piperidine in ethanol (25 mL)was stirred at room temperature for 1h. The reaction mixture was then poured onto water (200 mL) and set aside for an overnight. The precipitated solid product was collected by filtration, washed with water, dried and recrystallized from the appropriate solvent.

1(R=4-$CH_3C_6H_4$): Recrystallized from ethanol as needles. (2.1g, 73%) m.p.113-115°C. ν_{max} (cm^{-1}, KBr): 2224 (CN), 1710 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.30 (t,3H, CH_3); 2.34 (s, 3H, CH_3); 4.20 (s, 2H, CH_2); 7.18 - 7.62 (m,4H,Ar H); 8.10(s, 1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.2(CH_3);117.72(CN); 21.32 (CH_3),60.92(CH_2); 102.72, 154.60 (Olefinic C); 128.52, 128.90, 129.14, 137.69 (ArC) 162.14(CO). Anal.%Calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51. Found: C, 72.51; H, 6.06; N, 6.49.

2(R=4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles. (2.1g, 73%) m.p120-122 C. ν_{max} (cm^{-1}, KBr): 2235 (CN), 1708 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.29 (t,3H, CH_3); 3.81 (s, 3H, OCH_3); 4.26 (s, 2H, CH_2); 7.11 - 7.97 (m,4H,Ar H); 8.11(s, 1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.20(CH_3);55.81(CH_3O),117.71(CN); 60.90(CH_2); 102.71, 154.59 (Olefinic C); 114.22,124.42,13019,159.80 (ArC) 162.12(CO). Anal.%Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.43; H, 5.75; N,6.16.

3(R= 4-$(CH_3)_2NC_6H_4$: Recrystallized from ethanol as needles. (2.5g, 75%) m.p.110-112°C. ν_{max} (cm^{-1}, KBr): 2228 (CN),1718 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.29 (t,3H, CH_3); 3.06 (s, 6H, CH_3); 4.22 (s, 2H, CH_2); 6.66-8.23 (m,4H,Ar H); 7.91(s,1H, olefinic CH).

^{13}CNMR (δ/ppm, DMSO-d_6):14.10(CH_3), 41.37 (CH_3), 60.94 (CH_2), 117.54 (CN), 102.76,154.34 (Olefinic C), 111.12,119.02, 133.54, 150.28, (ArC) 163.12(CO). Anal.%Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.81; H, 6.62; N, 11.45.

4(R=4-BrC_6H_4): Recrystallized from ethanol as needles. (2.1g, 73%) m.p.118-120°C. ν $_{max}$ (cm^{-1}, KBr): 2221 (CN), 1710 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.30 (t,3H, CH_3); 2.34 (s, 3H, CH_3); 4.26 (s, 2H, CH_2); 7.61 - 7.77(m,4H,Ar H); 8.09(s, 1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.22(CH_3);117.80(CN); 60.87(CH_2); 102.69, 154.58 (Olefinic C); 122.32,131.34,131.58,132.3 (ArC) 162.25(CO). Anal.%Calcd for $C_{12}H_{10}BrNO_2$: C, 51.45; H, 3.60; N, 5.00. Found: C, 51.60; H, 3.75; N,6.49.

5(R=4-OH-3-$CH_3OC_6H_3$): Recrystallized from methanol as needles. (2.2g, 72%) m.p.95-97°C.ν $_{max}$ (cm^{-1}, KBr): 2230 (CN), 3226 (OH), 1720 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H, CH_3O); 5.35 (s, 1H, OH) ; 7.00-7.78 (m,3H,Ar H); 8.12 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.23(CH_3), 56.13 (CH_3O),60.84(CH_2),117.71(CN),111.78,116.76,122.85,128.73, 147.68, 149.22 (ArC)162.83(CO). Anal.%Calcd for $C_{13}H_{13}NO_4C$, 63.15; H, 5.30; N, 5.67. Found: C, 63.17; H, 5.33; N, 5.70.

6(R=4, 3-(OCH_2O)C_6H_3): Recrystallized from ethanol as needles. (2.1g, 73%) m.p.115-117°C. ν $_{max}$ (cm^{-1}, KBr): 2221 (CN), 1710 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.31 (t,3H, CH_3); 4.20 (s, 2H, CH_2); 6.06(s,2H,CH_2),6.94-7.32 (m,3H,Ar H); 8.23(s, 1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.25(CH_3);117.76(CN); 60.91(CH_2);161.53,81.50(Olefinic C); 101.22(OCH_2O),108.4,111.51,122.55,128.61,148.00,148.71 (ArC) 162.11(CO). Anal.%Calcd for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.72; H, 4.48; N, 5.70.

7(R= C_6H_4CH=CH): Recrystallized from ethano/methano as needles. (2.7g, 78%) m.p.98-100°C. ν $_{max}$ (cm^{-1}, KBr): 2226 (CN),1714(CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.30(t,3H, CH_3); 4.26 (s, 2H, CH_2); 6.71 (s, 1H, olefinic CH); 7.08 (s, 1H, olefinic CH); 7.39-7.60 (m,5H,Ar H); 8.05 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.21(CH_3), 60.88 (CH_2), 103.23,125.20,134.76,138.42(Olefinic C), 112.73, (CN), 127.93, 128.52, 128.64, 135.28 (ArC) Anal.% Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 73.96; H, 5.75, N, 6.19

8(R=2-Theinyl): Recrystallized from ethanol as needles. (2.6g, 77%) m.p.90-92°C. (ν_{max} (cm^{-1}, KBr):), 2222 (CN),1715 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.29 (t,3H, CH_3); 4.22 (s, 2H, CH_2);7.52-8.00 (m,3H,Ar H); 8.18 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.23(CH_3), 60.82 (CH_2), 89.42, 156.77 (Olefinic C), 117.68 (CN), 128.34, 129.15, 130.36,

137.81 (ArC),163.15. Anal.%Calcd for $C_{10}H_9NO_2S$: C, 57.95; H, 4.38; N, 6.76. Found: C, 57.97; H, 4.40; N, 6.74.

9(R=2-Furyl): Recrystallized from ethanol as needles. (2.6g, 77%) m.p.95-98°C. (ν_{max} (cm^{-1}, KBr):), 2222 (CN),1715 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.29 (t,3H, CH_3); 4.23 (s, 2H, CH_2);6.85-8.16 (m,3H,Ar H); 8.22 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 14.22(CH_3), 60.90 (CH_2), 93.61, 157.02 (Olefinic C), 117.70 (CN), 109.45,112.72,143.75,151.49(ArC),163.15. Anal.%Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.75; N, 7.33. Found: C, 62.93; H, 4.61; N, 7.42

10 (R=4-$CH_3C_6H_4$): Recrystallized from ethanol as needles. (3.4g,86%) m.p.128-130 °C. ν_{max} (cm^{-1}, KBr): 2220 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.34 (s, 3H, CH_3); 7.18-7.59 (m,4H,Ar H); 7.79 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 21.35 (CH_3), 81.44, 161.58 (Olefinic C);113.62 (CN);128.42; 128.54, 128.92, 137.64 (ArC). Anal.%Calcd for $C_{11}H_8N_2$: C, 78.55; H, 4.79; N, 16.66. Found: C, 78.58; H, 4.76; N, 16.69.

11 (R =4-$CH_3OC_6H_4$): Recrystallized from methanol as needles. (4.1g, 91%) m.p.106-108 °C. ν_{max} (cm^{-1}, KBr): 2233 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.88 (s,3H, CH_3O); 6.89-7.60 (m,4H,Ar H); 7.97 (s,1H, Olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.83 (CH_3O), 113.9 (CN), 81.49, 161.59 (Olefinic C), 114.56, 123.84, 130.22, 159.81 (ArC). Anal.%Calcd for $C_{11}H_8N_2O$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.70; H, 4.40; N, 14.35.

12 (R= 4-$(CH_3)_2NC_6H_4$): Recrystallized from ethanol as needles. (2.8g, 78%) m.p.158-160°C.ν_{max} (cm^{-1}, KBr): 2225 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.06 (s, 6H, $2CH_3$); 6.71-7.72 (m,4H,Ar H); 8.09 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 41.37 (CH_3), 113.55 (CN), 81.40, 161.61 (Olefinic C), 111.74, 120.94, 129.72, 150.32 (ArC). Anal.%Calcd for $C_{12}H_{11}N_3$: C, 73.07; H, 5.62; N, 21.30. Found: C, 73.05; H, 5.65; N, 21.28.

13 (R= 4-BrC_6H_4): Recrystallized from ethanol as needles. (2.8g, 78%) m.p.158-160°C.ν_{max} (cm^{-1}, KBr): 2225 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 7.51-7.73 (m,4H,Ar H); 7.89 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 113.62 (CN), 81.42, 161.50 (Olefinic C), 122.33,128.62,130.42,131.51 (ArC). Anal.%Calcd for $C_{10}H_5BrN_2$: C, 51.53; H, 2.16; N, 12.02. Found: C, 51.62; H, 2.24; N, 12.12.

14(R=4-CH_3O-3-OH-C_6H_4): Recrystallized from ethanol as needles. (3.1g, 82%) m.p.118-119°C. ν_{max} (cm^{-1}, KBr): 2228 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s, 3H, CH_3O) 5.33 (s, 1H, OH); 6.99-7.18 (m,3H,Ar H); 8.12 (s,1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6): 56.13 (CH_3O), 113.7 (CN), 81.50,161.30 (Olefinic C), 111.93,116.82, 122.92, 128.84,

147.92, 149.15 (ArC). Anal.%Calcd for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N, 13.99. Found: C, 65.98; H, 4.05; 14.02.

15((R=4, 3-(OCH_2O)C_6H_3):(R=4, 3-(OCH_2O)C_6H_3): Recrystallized from ethanol as needles. (2.1g, 73%) m.p.115-117°C. ν_{max} (cm^{-1}, KBr): 2221 (CN), 1710 (CO). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.10(s,2H,CH_2),6.93-7.30 (m,3H,Ar H); 8.00(s, 1H, olefinic CH). ^{13}CNMR (δ/ppm, DMSO-d_6):;113.63(CN); 161..51,81.42 (Olefinic C); 101.21(OCH_2O),108.52,111.60,122.50,128.52,148.02,148.72 (ArC). Anal.%Calcd for $C_{11}H_6N_2O2$: C, 66.67; H, 3.05; N, 14.14. Found: C, 66.72; H, 3.21; N, 14.54.

16(R= C_6H_4CH=CH): Recrystallized from ethanol/methanol as needles. (2.7g, 77%) m.p.105-106°C. ν_{max} (cm^{-1}, KBr): 2216 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.71, 7.02, 7.79 (s,3H, olefinic CH); 7.35-7.66 (m,5H,Ar H).^{13}CNMR (δ/ppm, DMSO-d_6): 112.80 (CN),82.94,122.32,150.56,160.22 (Olefinic C), 127.94, 128.59, 128.62, 135.24 (ArC). Anal.%Calcd for $C_{12}H_8N_2$: C, 79.98; H, 4.47; N, 15.55. Found: C, 80.00; H, 4.50; N, 15.57.

3.1. In Vitro MTT Cytotoxicity Assay.

The synthesized compoundswere investigated for their *in vitro* cytotoxic effect via the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [24,25] against a panel of three human tumor cell lines, namely, Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, and colon carcinoma HT29 and a normal nontransformed human foreskin fibroblast Hs27 cell line. The procedures were done in a sterile area using a laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch-cultured for 10 days and then seeded at concentration of 10 × 103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37∘C for 24 h under 5% CO2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, freshmedium(without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of 100-50-25-12.5-6.25-3.125-1.56-0.78 μg/mL.DMSOwas employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL Penicillin Potassium, 10,000 μg/mL Streptomycin Sulphate, and 25 μg/mL Amphotericin B), and 1% L-Glutamine in96-well flat bottom microplate at 37∘C under 5% CO2.After 24 h of incubation, the medium was aspirated and

40 μL of MTT salt (2.5μg/mL) was added to each well andincubated for further 4 h at 37∘C under 5% CO2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37∘C. The absorbance was then measured using a microplate multiwall reader (Bio-Rad Laboratories Inc., model 3350, Hercules,California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented inTable 1 as LC50 (μM) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

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