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Synthesis of Some Quinoline 3-carbonitrile Derivatives and Their Biological Evaluation as Cytotoxic Agent

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Abstract: A series of 2-Amino-4-subistituted-5,67,8-tetrahydroquinoline-3-carbonitriles supported with some functionalities reported to contribute to significant chemotherapeutic potential were synthesized and evaluated for their cytotoxic activity. Ten compounds exhibited cytotoxic potential against a panel of three human tumor cell lines. Compounds **13,14** and**17**proved to be the most active agents with a broad spectrum of cytotoxic activity. Analog **14** was considered as the most active cytotoxic agent, being about two times more active than doxorubicin against the colon HT29 carcinoma cell line.

Keywords: Synthesis,Quinoline-3-carbonitriles, Cytotoxic

1. Introduction

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of death after cardiovascular diseases and is characterized by unregulated proliferation of cells. Therefore, such rapid spread of cancer has stimulated an unprecedented level of medicinal chemistry research activity directed towards the search for new structure leads that may be of use in designing novel antitumor drugs. In this view, much interest has been focussed on pyridines and pyridine fused-ring systems since they are proved to be biological versatile compounds possessing variety of activities. Among these, wide range of chemotherapeutic activities have been ascribed to pyridine derivatives including the antimicrobial[1-3], antitubercular [4,5], antiamoebic[6], antiparasitic[7,8], antiviral [9.10]. Moreover, particular interest has been focussed on cyanopyridine derivatives owing to their well documented anticancer[11-15]activities. Motivated by these facts, it was thought worthwhile to synthesize and investigate the anticancer and antimicrobial activities of some new hydroxyl- and amino-cyanopyridine derivatives. Furthermore, some structure hybrids comprising both the pyridine and some

biologically active rings such as aryl or theinyl moieties, in one and the same structure entity. This combination is suggested in an attempt to investigate the influence of such hybridization on the anticipated anticancer and/or antimicrobial activity, hoping to discover a new structure lead that would have a remarkable biological significance. The target compounds were rationalized so as to comprise the pharmacophores and functionalities that are believed to be responsible for the biological significance of some relevant anticancer agents. The substitution pattern of such derivatives was selected so as to confer different electronic environment to the molecules that would affect their pharmacokinetics.

2. Results and discussion

2.1 Chemistry

Treatment of the appropriate cycloalkane derivative with the arylidene malononitrile derivatives**1**in the presence of ammonium acetate afforded the corresponding 2-Amino-4- quinoline-3-carbonitriles **2-5**or the 2-amino-4- subistituted -6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridine**6**respectively, in good yields.

12-6

Scheme 1

2:R=4-$CH_3C_6H_4$, R'=H, n=1; 3:R=4-$CH_3OC_6H_4$, R'=H, n=1;4:R=4-$CH_3C_6H_4$, R'=CH_3, n=1;5:R=4-$CH_3OC_6H_4$, R'=CH_3, n=1;6:R=4-$CH_3OC_6H_4$,R'=H, n=2

The formation of the above 2-aminotetraquinolines may be explained according to the following mechanism: The reaction seemed to be started by first addition of active hydrogen of compound **A** to the ethylenic double bond of

compound **B** to give **C**. Ammonia was added to the nitrile group in **C** to give **D** which loss a molecule of water to yield **E**, which in turn was converted to the final product by auto-oxidation.

The IR spectra of the 2-aminotetraquinolines**2-6**exhibited absorption bands within the range of 2215 - 2220cm^{-1} and 3329-3394 cm^{-1} for the CN and NH_2 groups respectively. Their structure was further confirmed from their ^{1}H NMR and ^{13}CNMR which showed expected numbers of protons and as well as the expected number of aromatic and aliphatic carbons respectively.Reaction the appropriate 3-cyanoquinolinederivatives **2-6** with formic acid resulted in the formation of thecorrespondingtetrahydropyrimido[4,5-b]quinolin-4(3H)-ones **7-9** (Scheme 2). Their IR spectra were characterized by the absence of the CN group absorption and the appearance of new sharp absorption bands at 1646-1655 cm^{-1} due to the new C=O groups as well as an NH absorption bands at 3282-3342 cm^{-1}. Their ^{1}H NMR which showed beside the aromatic protons an exchangeable singlet of one proton intensity at δ 7.81-8.20 for the NH group. The structures were further supported by ^{13}C NMR spectral data which showed the expected number of carbons signals (see experimental section). On the other hand, treatment the appropriate appropriate 2-Aminoquinolinederivatives **1-6**with acetic anhydride in presence of few drops of concentrated sulphuric acid gave the corresponding 2-methyl-tetrahydropyrimido[4,5-b]quinolin-4(3H)-ones

10-12. Their IR spectra of lacked the CN bands exists in the starting pyridines and exhibited a carbonyl absorption bands at 1652-1657 cm^{-1}. The ^{1}H-NMR spectra showed beside the aromatic protons an exchangeable singlet of one proton intensity at δ 8.02-8.17due to the NH group as well as a singlet of three proton intensity at δ2.21-2.44 ppm due to the new CH_3 group introduced in position-2. Moreover, their ^{13}C NMR spectral data exhibited beside the expected number of aliphatic and aromatic carbons, a new singlet at δ 20.19-21.40 ppm due to the CH_3 group as well as a CO signal at δ171.80-172.39.

Condensation of the original compounds **1-6**with phenyl isothiocyanate in pyridine medium afforded the corresponding N-phenylthiocarbamoyl analogs **13-15**. The IR spectra of these compounds showed C=S absorption at 1216-1229 cm^{-1} as well as an NH absorptions in the regions 3322-3374 cm^{-1}. The structures were further supported from their ^{1}H NMR which showed the aromatic protons and two exchangeable singlets each of one proton intensity at the regions 9.01-9.25 and 7.89-8.16 for the NH and =nh respectively. Further confirmation for the structure arises from their ^{13}C NMR spectral data which exhibited the expected number of aliphatic and aromatic carbons as well as a thiocarbony signal at δ179.82-182.83. Furthermoreheating the key inter mediates**1-6** with formamide,afforded the corresponding pyrimido-quinoline derivatives**16-18** in good yields. The IR spectra of these compounds exhibited NH and NH_2 absorbtion bands in the region3263-3393cm^{-1}. Their ^{1}H NMR which showed beside the aromatic protons an exchangeable singlet of one proton intensity at δ 7.83-8.15 for the NH group. The structures were further supported by ^{13}C NMR spectral data which showed the expected number of carbons signals (see experimental section).

Scheme 2

2.2 In VitroMTT Cytotoxicity Assay.

All the synthesized compounds were evaluated for their *in vitro* cytotoxiceffect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method[16,17] againsta panel of three human tumor cell lines, namely, coloncarcinoma HT29, hepatocellular carcinoma HePG2, andCaucasian breast adenocarcinoma MCF7. The results arepresented in Table 1 as LC50 (μM) which is the lethalconcentration of the compound that causes death of 50% ofthe cells in 24 h.The obtained data revealed that the three tested humantumor cell lines exhibited variable degree of sensitivity profilestowards ten of the tested compounds, namely,**2,3,7,8,10,11,13,14,16** and **17** whereas the rest compoundswere either marginally active or even totally inactive.Regarding the activity against the human colon carcinomaHT29, this cell line proved to be very sensitive to all theten active compounds. In particular, itrevealed distinctivesensitivity towards compounds **13,14 and 17**(LC50 24.6,22.4 and 29.3μM, resp.) even higher than doxorubicin (LC5040.0 μM), thereference standard cytotoxic agent utilized inthis assay. Meanwhile, compounds **11**and **16**(LC50 49.4 and42.4μM, resp.) were nearly equipotent to doxorubicin (LC5040.0 μM), whereas compounds **7,8** and **10**(LC50 70.5, 61.3 and70.3μM, resp.) showed moderate cytotoxic potential againstthe same cell line. Shifting to the hepatocellular carcinomaHepG2, this cell line showed mild

to weak sensitivity towardsseven of the tested analogs with LC50 range 54.4-112.2 μM,when compared to doxorubicin (LC50 3.0 μM). Among these,the highest activity was displayed by compounds **13,14** and **17**(LC50 60.5,54.4 and 71.2μM, resp.). On the other hand,the human breast cancerMCF 7 emerged as the least sensitiveamong the cell lines tested as its growth was affected by thepresence of only five test compounds. However, a remarkablegrowth inhibition potential was shown by analogs **13,14 and 17**asevidenced from their LC50 values (LC50 9.4,8.1 and10.2μM, resp.),which represents about 40–60%of the activityof doxorubicin (LC50 4.0 μM). Further interpretation of the results revealed that compounds**13,14,16 and 17**showed considerable broad spectrumcytotoxic activity against the three tested human tumorcell lines. In particular, compounds **13,14 and 17**provedto be the most active members in this study with specialeffectiveness against both the colon carcinoma HT29 (almosttwice as active as doxorubicin; LC50 24.6,22.4 and29.3 versus40 μM, resp.) and human breast cancer MCF 7 (about 40–60% of the activity of doxorubicin; LC50 9.4,8.1, and 10.2versus 4.0 μM, resp.).

A close examination of the structures of the activecompounds showed that the nature of substituent (R), together with ring entity (mono- or bicyclic), seemed toinfluence the cytotoxic activity. In this context, compoundssubstituted with the **4-$CH_3OC_6H_4$** counterpart (**3,8,11,14 and 17**) were in favor ofbetter cytotoxic activity, when compared with their **4-$CH_3C_6H_4$**congeners (**2,7,10,13 and 16**), as revealed from theirLC50 values in Table 1. Moreover, the bicyclic pyrido[2,3-*d*]pyrimidines proved to be more active than the monocyclicnicotinonitriles. In this view, although the starting nicotinonitriles**2** and **3**lacked or have weakcytotoxic efficacy, yet the bicyclic pyrido[2,3-d]pyrimidine derivatives**7,810,11,13,14,16 and 17**showed overall mild to moderateactivity, among which analog **14**(R = **4-$CH_3OC_6H_4$**)was relatively themost activeregardingbothpotency and spectrum. However, cyclization of the appropriate nicotinonitriles with phenyl isothiocyanate yielded the two remarkable active analogs, namely, **13 and 14**.

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

Compd no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
2	148.4	112.2	-[b]
3	130.5	-	-
7	70.5	-	-
8	61.3	109.5	-
10	70.3	111.5	-
11	49.4	91.8	86.4
13	24.6	5	9.4
14	22.4	54.4	8.1
16	42.4	-	20.6
17	29.3	71.2	10.2
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.

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. 1H and 13C NMR spectra were recorded on a Bruker WM-600 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-*d6* 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.

2-Amino-4-subistituted-5,67,8-tetrahydroquinoline-3-carbonitriles (2&3), 2-Amino-8-methyl-4-subistituted-5,67,8-tetrahydroquinoline-3-carbonitriles (4&5) and 2-amino-4- subistituted -6,7,8,9-tetrahydro-5H-cyclohepta[b]pyridine-3-carbonitrile (6)

A mixture of appropriate cyclic ketone (10mmol), arylidene malononitril derivative **1**(10 mmol) and excess ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (30 mL) was heated under reflux for 4-6 h.The progress of the reaction was monitored by TLC. After complete conversion, as indicated by TLC,the solid material which separated during heating was collected by filtration and recrystallized from a mixture of appropriate solvent as needles.

2: Rrecrystallized from ethanol as needles. (2.02g, 73%) m.p.240-242°C. $\nu_{max.}$ (cm^{-1}, KBr):3415, 3173(NH_2), 2224(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.53-2.84 (4m,8H, Cyclohexyl H); 2.38 (s, 3H, CH_3); 6.05 (s,2H,NH_2), 7.11-7.25 (m,4H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6):21.32(CH_3), 22.34, 24.51, 28.32, 33.9 (cyclohexylC), 115.61(CN), 87.51, 116.62, 121.12, 128.02, 129.45, 133.64, 151.45, 154.71, 162,62 (Ar C). Anal.% Calcd for $C_{17}H_{17}N_3$: C, 77.54; H, 6.51; N, 15.96. Found: C, 77.48; H, 6.72; N, 16.10.

3:Rrecrystallized from ethanol as needles. (2.02g, 73%) m.p.218-220°C. $\nu_{max.}$ (cm^{-1}, KBr):3405, 3210(NH_2), 2221(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.39-2.48 (4m,8H, Cyclohexyl H); 3.81 (s, 3H, OCH_3); 6.08 (s,2H,NH_2), 7.08-7.21 (m,4H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 22.70,23.12,24.11, 29.12 (cyclohexylC),55.80(CH_3O) 115.31(CN), 87.42, 113.71,125.40,126.22,129.60,152.00,159.81,160.21,161.11 (Ar C). Anal.% Calcd for $C_{17}H_{17}N_3O$: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.22; H, 6.28; N, 15.10.

4: Rrecrystallized from ethanol as needles. (2.02g, 73%) m.p.210-212°C. $\nu_{max.}$ (cm^{-1}, KBr):3416, 3186(NH_2), 2220(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.33 (d J=10Hz, 3H ,CH_3); 1.47- 3.23(4m,7H, Cyclohexyl H); 2.33 (s, 3H, CH_3); 6.16 (s,2H,NH_2), 7.24-7.54 (m,4H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 17.75(CH_3), 20.91(CH_3), 18.9, 24.51, 28.32, 33.9 (cyclohexylC), 114.61(CN), 86.11,

115.92, 120.14, 128.32, 129.56, 133.77, 150.65, 156.01, 162,82 (Ar C). Anal.% Calcd for $C_{18}H_{19}N_3$: C, 77.95; H, 6.90; N, 15.15. Found: C, 78.08; H, 6.72; N, 15.40.

5: Rrecrystallized from ethanol as needles. (1.9g, 68%) m.p.177-179°C. ν $_{max.}$ (cm^{-1}, KBr):3410, 3175(NH_2), 2218(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.35 (d J=10Hz, 3H ,CH_3); 1.68, 2.39, 2.88 (4m,7H, Cyclohexyl H); 6.12 (s,2H,NH_2), 7.10-7.26 (m,4H,ArH) ;3.75(s,3H,OCH_3). ^{13}CNMR (δ/ppm, DMSO-d_6): 17.8(CH_3), 18.99, 24.56, 28.35, 33.98(cyclohexylC), 56.04(OCH_3), 114.65(CN), 86.08, 115.94, 120.23, 128.36, 129.73, 133.84, 150.72, 156.08 ,162,95 (Ar C). Anal.% Calcd for $C_{18}H_{19}N_3O$: C, 73.70; H, 6.53; N, 14.32. Found: C, 73.42; H, 6.71; N, 14.09.

6:Rrecrystallized from ethanol as needles. (2.02g, 73%) m.p.220-223°C. ν $_{max.}$ (cm^{-1}, KBr):3415, 3265(NH_2), 2222(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.67-2.85(4m,10H, Cycloheptyl H); 3.81 (s, 3H, OCH_3); 6.08 (s,2H,NH_2), 7.07-7.18 (m,4H,ArH). ^{13}CNMR (δ/ppm, DMSO-d_6): 26.21,28.41,28.71,32.63,36.12 (cycloheptylC), 55.80(CH_3O) 115.09(CN), 87.22, 113.58,125.34,126.51,129.18,151.87,159.34,160.78,161.43 (Ar C). Anal.% Calcd for $C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.32. Found: C, 73.72; H, 6.35; N, 14.24.

9-Methyl-5-substituted -6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H)-one (7&8) and 5-Substituted-3,6,7,8,9,10-hexahydro-4H-cyclohepta[5,6]pyrido[2,3-d]- pyrimidin-4-one (9)

A mixture of the appropriate tetrahydroquinoline derivative (10 mmol) and formic acid (5 ml) was heated in a boiling water bath for 30 min. After being cooled to room temperature, the reaction mixture was poured onto ice-cold water, the precipitated solid product was filtered, washed with water, dried and recrystallized from the appropriate solvent.

7: Rrecrystallized from methanol as needles. (2.0g,70%) m.p.137-139 °C. ν $_{max}$ (cm^{-1}, KBr):3252(NH), 1668 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.30(d J=10Hz, 3H ,CH_3); 2.37(s, 3H ,CH_3); 1.53- 2.67 (4m,7H, Cyclohexyl H); 6.84-7.32(m,4H,ArH); 7.89 (s, 1H,NH) . ^{13}CNMR (δ/ppm, DMSO-d_6): 17.52(CH_3), 21.21 (CH_3), 21.56, 28.29, 30.34, 35.99 (cyclohexyl C), 88.20,

116.52, 118.24, 122.17, 130.05, 131.50, 135.67, 152.43, 157.82, 164.90 (ArC), 170.99 (CO). Anal.% Calcd for $C_{19}H_{19}N_3O$: C, 74.73; H, 6.27 ; N, 13.76. Found: C, 74.51 ; H, 6.47 ; N, 13.53.

8: Rrecrystallized from ethanol as needles.(2.3g, 72%) m.p.186-188 °C. ν $_{max}$ (cm^{-1}, KBr):3247(NH), 1670 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.36(d J=10Hz, 3H ,CH_3); 3.73(s, 3H, OCH_3); 1.52- 2.72 (4m,7H, Cyclohexyl H); 6.97-7.26(m,4H,ArH); 7.91 (s, 1H,NH) . ^{13}CNMR (δ/ppm, DMSO-d_6): 17.9(CH_3), 21.52, 28.35, 30.43, 35.96 (cyclohexyl C), 56.02(OCH_3), 91.87, 116.51, 118.22, 122.18, 130.05, 131.56, 135.67, 152.44, 157.89, 164.92 (ArC), 171.06 (CO). Anal.% Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96 ; N, 13.07.Found: C, 71.32 ; H, 5.73 ; N, 12.91.

9: Rrecrystallized from ethanol as needles.(2.3g, 72%) m.p.220-223 °C. ν $_{max}$ (cm^{-1}, KBr):3265(NH), 1671 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.73(s, 3H, OCH_3); 1.75-3.10 (3m,10H, Cycloheptyl H); 7.01-7.72(m,4H,ArH); 8.12 (s, 1H,NH) . ^{13}CNMR (δ/ppm, DMSO-d_6):26.23,28.41,28.71,32.71,37.78 (cyclohexyl C), 55.82(OCH_3), 114.81,119.72,129.56,130.21,135.08,145.76,151.91,152.52,156.70,161.12(ArC), 169.12 (CO). Anal.% Calcd for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96 ; N, 13.07.Found: C, 71.24; H, 6.08 ; N, 13.02.

2 2,9-Dimethyl-5-substituted -6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H)-ones (10&11) and 2-Methyl-5-2ubstituted-3,6,7,8,9,10-hexahydro-4H-cyclohepta[5,6]- pyrido[2,3-d]- pyrimidin-4-one (12)

A mixture of the appropriate tetrahydroquinoline derivative(10 mmol), acetic anhydride (5 ml) and conc. H_2SO_4 (0.5 ml) was heated in a boiling water bath for 10 min, then cooled, poured onto ice-cold water, treated with 20% NaOH solution till alkaline (pH 11), the crude solid product was filtered , dried and recrystallized from the appropriate solvent.

10: Rrecrystallized from ethanol as needles. (1.66g, 52%) m.p.135-137°C. ν $_{max}$ (cm^{-1}, KBr):3170 (NH), 1705 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.12(s,3H ,CH_3); 2.35(s, 3H ,CH_3); 1.36(d J=10Hz, 3H ,CH_3); 1.63, 1.73, 2.24, 2.68 (4m,7H, Cyclohexyl H); 7.01-7.59 (m,4H, ArH); 8.14 (s,1H,NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 19.82(CH_3), 20.86(CH_3), 21.68 (CH_3), 22.21, 26.63, 30.8, 35.66 (cyclohexyl C), 88.24, 116.23, 118.83, 122.73, 130.45, 131.22, 135.18, 151.99, 157.26,

164.14 (ArC), 172.35 (CO). Anal.% Calcd for $C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.16. Found: C, 75.41; H, 6.47; N, 13.25.

11: Rrecrystallized from ethanol as needles. (1.37g, 41%) m.p.122-124°C. ν_{max} (cm^{-1}, KBr):3175(NH), 1684 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.13(s, 3H ,CH_3); 1.39 (d J=10Hz, 3H ,CH_3); 1.62, 1.77, 2.22, 2.89 (4m,7H, Cyclohexyl H); 6.83-7.25 (m,4H,ArH); 8.12 (s,1H,NH) ; 3.74 (s, 3H, OCH3) . ^{13}CNMR (δ/ppm, DMSO-d_6): 18.34 (CH_3), 21.66 (CH_3), 22.14, 26.64, 30.91, 35.77 (cyclohexyl C), 56.01 (OCH_3), 88.25, 116.33, 118.95, 122.77, 130.48, 131.18, 135.16, 151.96, 157.18, 164.09 (ArC), 172.39 (CO). Anal.% Calcd for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53. Found: C, 71.54; H, 6.42; N, 12.70.

12: Rrecrystallized from ethanol as needles. (1.37g, 41%) m.p.178-180°C. ν_{max} (cm^{-1}, KBr):3275(NH), 1687 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6):2.42(s,3H,CH_3), 3.74(s, 3H, OCH_3); 1.73-3.11 (3m,10H, Cycloheptyl H); 7.11-7.70(m,4H,ArH); 8.02 (s, 1H,NH) . ^{13}CNMR (δ/ppm, DMSO-d_6): 21.41(CH_3),26.31,28.31,28.67,32.81,37.66 (cycloheptylC),55.80(OCH_3),114.90,119.76,129.43,130.32,135.32,145.89,151.65,152.45,156.90,161.23(ArC), 169.23 (CO). Anal.% Calcd for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53. Found: C, 71.54; H, 6.42; N, 12.70.

4-Imino-3-phenyl-5-substituted-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinoline-2(1H)-thione (13), 4-Imino-9-methyl-3-phenyl-5-substituted -3,4,6,7,8,9-hexahydropyri- mido[4,5-b]quinoline-2(1H)-thiones (14&15)and4-Imino-3-

A mixture of appropriate tetrahydroquinoline derivative(10 mmol), phenyl isothiocyanate (0.15 g, 15 mmol) in pyridine (15 ml) was refluxed for 4 h. After cooling, the solid product was filtered off, washed thoroughly with water, dried and recrystallized from the appropriate solvent.

13: Rrecrystallized fromethanol as needles. (3.34g, 76%) m.p.134-136°C. ν_{max} (cm^{-1}, KBr):3345(NH), 1651 (C=N), 1187 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.42-2.54 (4m,8H, Cyclohexyl H);2.35 (s, 3H ,CH_3); 6.84-7.70(m,9H,ArH); 7.95(s,1H,=NH),9.12(s,1H,NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 19.96, 24.23, 28.65, 30.45 (cyclohexyl C), 21.32(CH_3), 91.78, 109.90, 124.62,

124.92, 12543, 126.45, 128.62, 128.76, 138.33, 139.39, 149.32, 156.90 (ArC), 163.21 (C=NH), 183.28 (CS). Anal.% Calcd for $C_{24}H_{22}N_4S$: C, 72.33; H, 5.56; N,14.06. Found: C, 72.25; H, 5.67; N, 14.17.

14:Rrecrystallized fromethanol as needles. (3.34g, 76%) m.p.134-136°C. ν_{max} (cm^{-1}, KBr):3365(NH), 1641 (C=N), 1195 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.38 (d, 3H ,CH_3); 1.55, 1.73, 2.48, 2.65 (4m,7H, Cyclohexyl H);2.33 (s, 3H ,CH_3); 6.87-7.67(m,9H,ArH); 7.98(s,1H,=NH),9.01(s,1H,NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 17.35 (CH_3), 18.54, 24.34, 28.25, 30.26 (cyclohexyl C), 20.93(CH_3), 90.32, 109.81, 124.55, 124.87, 125.35, 126.58, 128.45, 128.87, 138.42, 139.32, 149.66, 156.71 (ArC), 163.72 (C=NH), 182.23 (CS). Anal.% Calcd for $C_{25}H_{24}N_4S$: C, 60.38; H, 4.43; N,11.74. Found: C, 60.25; H, 4.26; N, 11.71.

15:Rrecrystallized from ethanol as needles. (3.34g, 78%) m.p.134-136°C. ν_{max} (cm^{-1}, KBr):3341,14(NH), 1635 (C=N), 1203 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.31 (d J=10Hz, 3H ,CH_3); 1.52, 1.72-2.80 (4m,7H, Cyclohexyl H);3.83 (s, 3H ,OCH_3); 8.16(s,1H,=NH); 9.25 (s,1H,NH); 6.65-7.22(m,9H,ArH);^{13}CNMR (δ/ppm, DMSO-d_6): 17.42 (CH_3), 18.58, 24.4, 28.21, 30.15 (cyclohexyl C), 56.02(CH_3O),109.8, 124.54, 124.7, 125.57, 126.88, 128.83, 129.18, 138.32, 139.21, 149.65, 156.86 (ArC), 163.87 (C=NH), 179.82 (CS). Anal.% Calcd for $C_{25}H_{24}N_4OS$: C, 70.07; H, 5.64; N, 13.07. Found: C, 69.98; H, 5.66; N, 13.15.

4-Amino-9-methyl-5-substituted-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolins (16&17) and 4-Amino-5-substituted-4,6,7,8,9,10-hexahydro-3H-cyclohepta[5,6]- pyrido[2,3-d]pyrimidines (18)

A mixture of the appropriate tetrahydroquinoline (10 mmol) and formamide (10 ml) was heated under reflux for 2-3 h. The reaction mixture was cooled and the precipitated solid product was collected, washed with cold ethanol and recrystallized from the appropriate solvent.

16:Rrecrystallized from DMF/H_2O as needles. (2.37g, 78%) m.p.200-201°C. ν_{max} (cm^{-1}, KBr): 3358, 3410 (NH).^{1}HNMR (δ/ppm, DMSO-d_6): 1.34(d J=10Hz, 3H ,CH_3); 2.35(s,3H,CH_3), 1.63,

2.55, 2.94(4m,7H,Cyclohexyl H); 6.62(s,2H,NH_2); 7.12-7.37 (m,4H,ArH);8.15(s, 1H, H-2). ^{13}CNMR (δ/ppm, DMSO-d_6): 16.25 (CH_3), 20.92 (CH_3), 19.98, 25.16, 26.08, 29.45 (cyclohexyl C), 94.34, 111.54, 112.54, 113.78, 113.89, 121.98, 127.23, 127.65, 127.98, 149.35, 158.00(ArC). Anal.% Calcd for $C_{19}H_{20}N_4$: C, 74.97; H, 6.62; N,18.41. Found: C,75.13; H, 6.49; N;18.56.

17:Rrecrystallized from methanol as needles. (2.65g, 83%) m.p.121-123°C. ν $_{max}$ (cm^{-1}, KBr): 3360, 3489 (NH_2).^{1}HNMR (δ/ppm, DMSO-d_6): 1.35(d, 3H ,CH_3); 1.53, 1.65, 2.53, 2.68(4m,7H, Cyclohexyl H); 3.72(s, 3H, OCH_3), 6.52(s,2H, NH_2); 6.38-7.36 (m,4H,ArH);8.12(s, 1H, H-2). ^{13}CNMR (δ/ppm, DMSO-d_6): 15.65 (CH_3), 19.8, 24.75, 26.02, 29.34 (cyclohexyl C); 56.21(CH_3O), 94.28, 111.47, 112.54, 113.71, 113.85, 121.90, 127.12, 127.75, 127.92, 149.23, 157.02(ArC). Anal.% Calcd for $C_{19}H_{20}N_4O$: C, 71.23; H, 6.29; N,17.49. Found: C,71.12; H, 6.40; N;17.60.

18: Rrecrystallized from ethanol as needles. (1.37g, 41%) m.p.270-272°C. ν $_{max}$ (cm^{-1}, KBr):3371, 3445 (NH_2).^{1}HNMR (δ/ppm, DMSO-d_6): 1.73-3-08 (3m,10H, Cyclohexyl H); 3.74(s, 3H, OCH_3), 6.62(s,2H, NH_2); 7.02-7.69 (m,4H,ArH);8.15(s, 1H, H-2).. ^{13}CNMR (δ/ppm, DMSO-d_6): 26.51,28.56,28.49,32.91,37.54 (cycloheptylC),55.80(OCH_3),107.03,114.76,119.84,129.34,130.21,135.39,145.90,151.32, 152.34, 156.67, 161.31(ArC). Anal.% Calcd for $C_{19}H_{20}N_4O$: C, 71.23; H, 6.29; N,17.49. Found: C,71.41; H, 6.32; N;17.52

3.1. In Vitro MTT Cytotoxicity Assay.

The synthesizedcompoundswere investigated for their *in vitro* cytotoxic effectvia the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) method [16,17] against a panelof three human tumor cell lines, namely, Caucasian breastadenocarcinoma MCF7, hepatocellular carcinoma HepG2,and colon carcinoma HT29 and a normal nontransformedhuman foreskin fibroblast Hs27 cell line. The procedureswere done in a sterile area using a laminar flow cabinetbiosafety class II level (Baker, SG403INT, Stanford, ME,USA). Cells were batch-cultured

for 10 days and then seededat concentration of 10 × 103 cells/well in fresh completegrowth medium in 96-well microtiter plastic plates at 37∘Cfor 24 h under 5% CO2 using a water jacketed carbon dioxideincubator (Sheldon, TC2323, Cornelius, OR, USA). Mediawas aspirated, freshmedium(without serum) was added, andcells wereincubated either alone (negative control) or withdifferent concentrations of the test compounds to give a finalconcentration 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 testedcompounds and its final concentration on the cells was lessthan 0.2%. Cells were suspended in RPMI 1640 medium(for HepG2 and HT29 cell lines) and DMEM (for MCF 7cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mLPenicillin Potassium, 10,000 μg/mL Streptomycin Sulphate,and 25 μg/mL Amphotericin B), and 1% L-Glutamine in
96-well flat bottom microplate at 37∘C under 5% CO2.After 24 h of incubation, the medium was aspirated and40 μL of MTT salt (2.5μg/mL) was added to each well and
incubated for further 4 h at 37∘C under 5% CO2. To stopthe reaction and dissolve the formed crystals, 200 μL of10% sodium dodecyl sulphate (SDS) in deionized water wasadded to each well and incubated overnight at 37∘C. Theabsorbance was then measured using a microplate multiwall reader (Bio-Rad Laboratories Inc., model 3350, Hercules,California, USA) at 595 nm and a reference wavelength of620 nm. A statistical significance was tested between samplesand negative control (cells with vehicle) using independent*t*-test by SPSS 11 program. The results are presented in
Table 1 as LC50 (μM) which is the lethal concentration ofthe compound which causes death of 50% of the cells in24 h.

4. Reference

1. Abdel-Aziz, A. A., El-Subbagh, H. I., and Kunieda, T., *Bioorg. Med. Chem.*, **13** (2005) 4929-4935.

2. Aridoss, G., Balasubramanian, S., Parthiban, P., and Kabilan, S., *Eur. J. Med. Chem.*, 42 (2007) 851-860 .

3. Srivastava, B. K., Solanki, M., Mishra, B., Soni, R., Jayadev, S., Valani, D., Jain, M., and Patel, P. R., *Bioorg. Med. Chem.*, **15** (2007)1924-1929.

4. Mamolo, M. G., Falagiani, V., Vio, L., and Banfi, E., *Il Farmaco*, **54** (1999) 761-767.

5. Ranft, D., Seyfarth, T., Schaper, K.-J., Lehwark-Yvetot, G., Bruhn, C., and Buegem, A., *Arch. Pharm. Pharm. Med. Chem.*, **332** (1999) 427-430.

6. Pagliero, R. J., Lusvarghi, S., Pierini, A. B., Brun, R., and Mazzieri, M. R., *Bioorg. Med. Chem.*, **18** (2010) 142-150.

7. Abid, M., Husain, K., Azam, A., *Bioorg. Med. Chem. Lett.*, **15 (**2005) 4375-4379.

8. Goebel, T., Ulmer, D., Projahn, H., Kloeckner, J., Heller, E., Glaser, M., Ponte-Sucre, A., Specht, S., Sarite, S. R., Hoerauf, A,. Kaiser, A., Hauber, I., Hauber, J., and Holzgrabe, U., *J. Med. Chem.*,**51** (2008) 238-250.

9. Dragovich, P. S., Prins, T. J., Zhou, R., Johnson, T. O., Brown, E. L., Maldonado, F. C., Fuhrman, S. A., Zalman, L. S., Patick, A. K., Matthews, D. A., Hou, X., Meador, J. W., Ferre, R. A., and Worland, S. T., *Bioorg. Med. Chem. Lett.*, **12** (2002) 733-738.

10. Gudmundsson, K. S., Johns, B. A., Wang, Z., Turner, E. M., Allen, S. H., Freeman, G . A., Boyd Jr, F. L., Sexton, C. J., Selleseth, D. W., Moniri, K. R., and Creech, K. L. *Bioorg. Med. Chem.*, **13** (2005) 5346-5361.

11. Cocco, M. T., Congiu, C., Lilliu, V., and Onnis, V., *Eur. J. Med. Chem.*, **40** (2005) 1365-1372.

12. Ghorab M. M., Ragab F. A., Hamed M. M.,Eur. J. Med. Chem.,**44** (2009) 4211-4217.

13. Alqasoumi S. I., Al-Taweel A. M., Alafeefy A. M., Ghorab M. M., Noaman E., Eur. J. Med. Chem., **45** (2010) 1849-1853.

14. Liu Y.-Q., Yang L., Tian X., Med. Chem. Res., **16** (2007) 319-330.

15. Heiniger B., Gakhar G., Prasain K., Hua D. H., Nguyen T. A., Anticancer Res., **30** (2010) 3927-3932.

16. T.Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63,1983.

17. F. Denizot and R. Lang, "Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability," *Journal of Immunological Methods*, vol. 89, no. 2, pp. 271–277, 1986.