ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and Characterization of New Fused 4H-Pyranquinoline Carbonitrile Derivatives with Anticipated Antitumor Biological Activity.

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ABSTRACT

Quinoline moiety is versatile moiety for synthesis many drugs and anticipated highly pharmacological compounds.8-hydroxyquinoline reacts with *p*-methoxy or *p*-fluoro benzylidene malonitrile forming 4H-pyranoquinoline -3-carbonitrile derivatives; those undergoes cyclization *via* reacting with formic acid or formamide or using triethylorthoformate. The quinoline derivatives also reacts with ethyl or phenyl isothiocyanate forming corresponding thiourea derivatives that reacted with halogenated compounds yielding new thiazol pyranoquinoline-3-carbonotrile. The compounds were used to evaluate their antitumor potency on four human tumor cell lines namely; hepatocellular carcinoma *HepG2,* prostatic carcinoma *PC3*, colon carcinoma *HCT116* and lung carcinoma *A549*.

Keywords: Quinoline; carbonitrile, 4H-pyran, antitumor; malononitrile.

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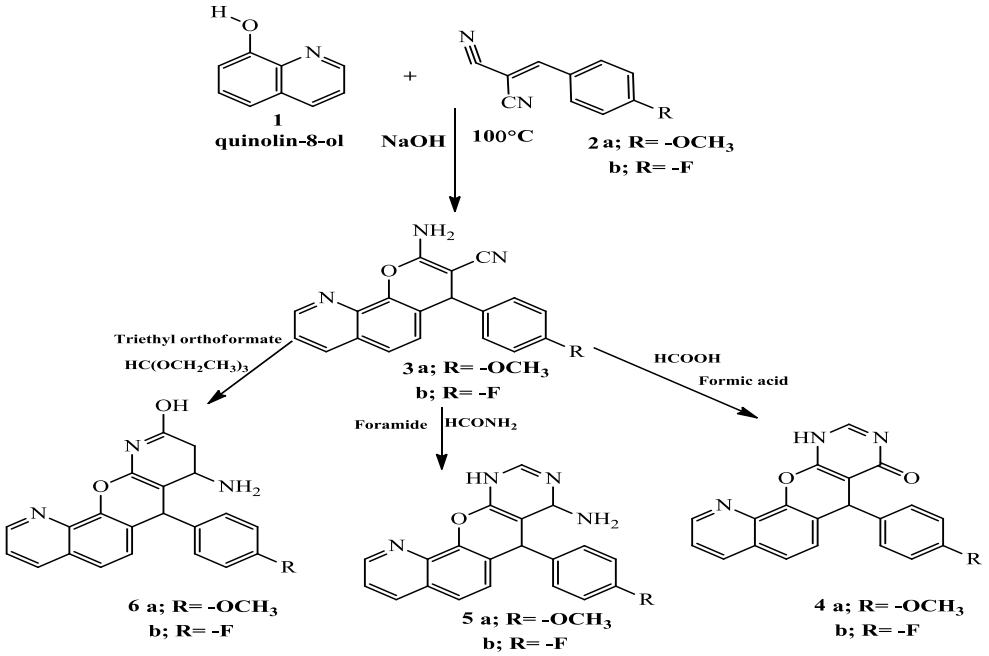
ISSN: 0975-8585

INTRODUCTION

Quinoline and pyran moieties are considered to be important chemical synthons of various physiological significances and pharmaceutical utilities quinoline ring containing compounds exhibit potent biological activities and has been proved by number of recent reports. Quinoline derivatives were synthesized and explored for their analgesic activity[1]as antiallergenic agents[2] in treating Alzheimer's disease (AD),[3] as anticancer[4,5] antitinephritic[6] antitumor[7] and anti-inflammatory activities. Therefore, many researchers have synthesized this important class of compounds as target structures and evaluated their biological activities. Our thesis provides some lights as small contribution in depth view of work done so far on quinoline and its biological activities [8].

The 4 H-Pyran nucleuses is a fertile source of biologically important molecules possessing a wide spectrum of biological and pharmacological activities, such as antimicrobial [9], antiviral [10, 11], mutagenicity[12], antiproliferative[13], sex pheromone[14], antitumor[15] ,cancer therapy[16], and central nervous system activity [17]. Therefore, the synthesis of such compounds has attracted strong interest.

RESULTS AND DISCUSSION



Scheme(1)

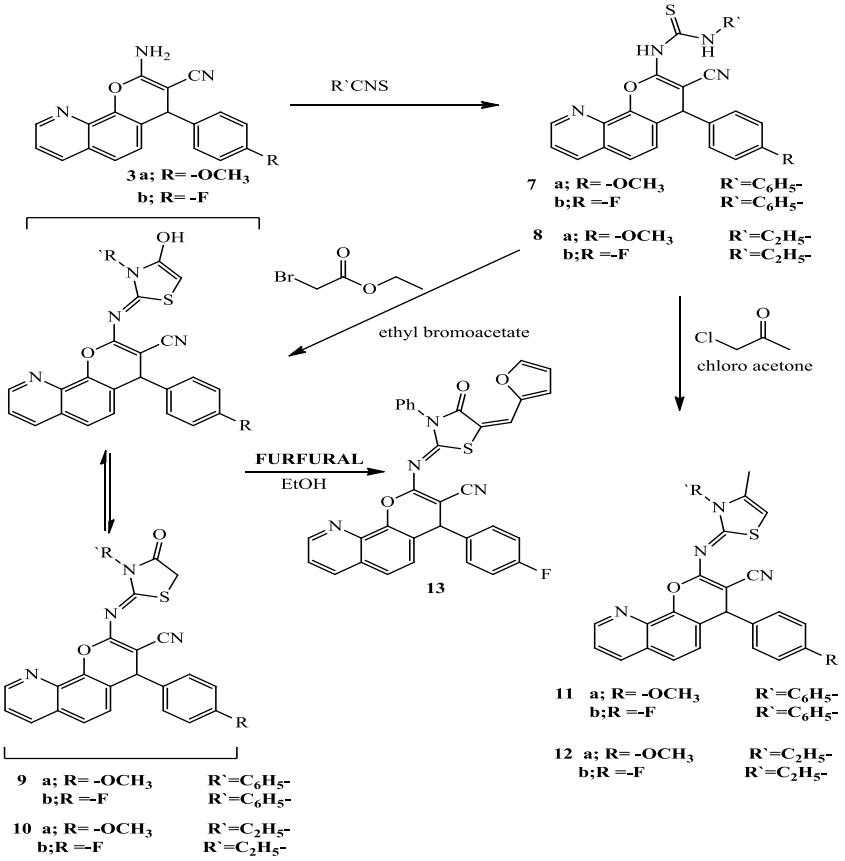
Herein we report a synthesis and characterization series of quinoline-3-carbonitrile derivatives. The versatile Starting compounds 3a,b were synthesized by Michael addition of 8-hydroxy quinoline on2-(4-methoxybenzylidene)malononitrile 2a or 2-(4-fluorobenzylidene) malononitrile2b in presence of sodium hydroxide *via* fusion. The 4H-pyrano [3, 2-h] quinoline-3-carbonitrile derivatives 3a, b undergoes cyclization by reaction with different reagents such formic acid to afford 4a ,b or using formamide to give 5a ,b or ethyl orthoformate forming 6a,b these derivatives showing disappearing of cyano group as in IR spectra and appearing new groups e.g., amidic CO ;NH&NH_2. (Scheme1).

Besides, the ethyl or phenyl isothiocyanate reacted with amino group of quinoline-3-carbonitrile derivatives 3a,b forming new thiourea derivatives 7a,b &8a,b that reacted with halogenated compounds such ethyl bromoacetate or chloroacetone affording 9a,b; 10a,b; 11a,b& 12a,b.

Compound (Z)-4-(4-fluorophenyl)-2-((4-hydroxy-3-phenylthiazol-2(3H)-ylidene) amino)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (9b)condensed with furfural forming 4-(4-fluorophenyl)-2-((Z)-((E)-5-(furan-2-

ISSN: 0975-8585

ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene)amino)-4H-pyrano[3,2-h]quinoline-3-carbonitrile(13) (Scheme2).



Scheme(2)

Antitumor study

The results of effect of the compounds on liver carcinoma (HepG2), colorectal carcinoma (HCT116), prostate carcinoma (PC3) and lung adenocarcinoma (A549) human cell lines showing in following (Table 1).

Table 1

Compound	LC50 (µM)			
	HepG2	HCT116	PC3	A549
6b	**169.6**	**175**	inactive	inactive
5a	inactive	inactive	inactive	inactive
7a	**142**	**161.4**	inactive	inactive
6a	**166.9**	**126.5**	inactive	inactive
8b	inactive	inactive	inactive	Inactive
3b	**113.4**	**151.3**	**122.9**	Inactive
7b	inactive	inactive	inactive	Inactive
4a	inactive	inactive	inactive	Inactive
4b	inactive	inactive	inactive	Inactive
8a	**inactive**	**57.6**	inactive	Inactive
5b	inactive	inactive	inactive	Inactive
3a	**106.3**	**100.2**	inactive	Inactive
2a	inactive	inactive	inactive	Inactive
2b	**377.6**	**273**	**209.1**	**377.6**
Doxrubicin (positive control)	**37.8**	**65.1**	**41.1**	**48.8**

LC50 = the concentration which kills 50% of the cells
The compounds were tested at 100ppm on BJ1 human fibroblast normal cell line.

ISSN: 0975-8585

The activity of the compounds on tumour cell skin lines and on normal cell lines at 100ppm is showing in the following Table (2).

Table 2

Compound	BJ1	HepG2	HCT116	PC3	A549
6b	13%	68%	**93%**	40%	0%
5a	56%	22%	37%	22%	0%
7a	22%	73%	**89%**	55%	0%
6a	54%	70%	**98%**	64%	25%
8b	10%	25%	17%	37%	0%
3b (315µM)	**90%**	**84%**	**96%**	**84%**	0%
7b	10%	30%	40%	54%	0%
4a	35%	44%	28%	34%	0%
4b (289.5µM)	**100%**	33%	36%	15%	0%
8a (240µM)	**100%**	67%	**90%**	68%	6%
5b	0%	50%	59%	72%	0%
3a (303.6µM)	**100%**	**80%**	**89%**	70%	40%
2a (542.9µM)	**100%**	19%	58%	22%	9%
2b (580.8µM)	**100%**	**93%**	**98%**	**93%**	**83%**

***In vitro* antitumor screening**

The synthesized compounds 3a,b ;4a,b;5a,b; 6a,b;7a,b;8a,b were subjected to in vitro antitumor screening against human cancer cell lines using cell based approach [18-21]. Test compounds were used to evaluate their antitumor potency on four human tumor celllines namely: hepatocellular carcinoma HepG2, prostatic carcinoma PC3, colon carcinoma HCT116 and lung carcinomaA549. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] to purple Formosan [22-23]. Aprobit analysis was carried for LC50 determination using SPSS 11program. The antitumor drug doxorubicin was used as a positive control. The in vitro antitumor screening was performed adopting previously reported procedures [21-23]. Cells were suspended in RPMI 1640 medium for HepG2 and DMEM forA549, PC3 and HCT116, 1% antibiotic-antimycotic mixture (10,000 u/ml potassium penicillin, 10,000 mg/ml streptomycin sulfate and 25 mg/ml amphotericin B) and 1% L-glutamine at 37 °C, under 5% CO_2 and 95%humidity. Cells were seeded at concentration of 10 x103 cells/well in fresh complete growth medium in 96-well micro titer plates for24 h. Media was aspirated, fresh medium (without serum) was added and cells were incubated with different concentrations of sample to give a final concentration of (100, 50, 25, 12.5, 6.25,3.125, 0.78 and 1.56 ppm.) 0.5% DMSO was used as negative control and doxorubicin was used as positive control. MTT assay was used for assessment of cytotoxicity [21-23]. After 48 hr of incubation, medium was aspirated, 40 µl MTT salt (2.5 mg/ml) were added to each well and incubated for further 4 h. To stop there action and dissolving the formed crystals, 200 µl of 10% sodium dodecyl sulfate (SDS) in deionized water were added to each well and incubated overnight at 37 °C. The absorbance was then measured at 595 nm and a reference wave length of 620 nm. The % cytotoxicity was calculated according to the formula:

[1-(OD compound/OD negative control)]_100. A probit analysis was carried for LC50 determination using SPSS11 program.

EXPERIMENTAL

General Remarks

All melting points are incorrect and measured in degree centigrade. Elemental analysis was carried out in the Microanalytical unit, National Research Center, Dokki, and Cairo. Infrared spectra were recorded on Matheson 5000 FTIR Spectrometer using HBr Wafer technique.^{1}H NMR spectra were determined on Varian-Gemini 200 MHz and Joel-Ex-270 MHz NMR Spectrometer using TMS as an internal standard with (chemical shift δ = 0 ppm). The purity of the synthesized compounds was tested by Tin-Layer Chromatography (TLC).Biological studies were performed in Pharmacognosy Department, National Research Center (NRC), and Cairo, Egypt.

Synthesis of 2-amino-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (3a)

A mixture of 8-hydroxy quinoline (0.01 mol, 1.45 g); sodium hydroxide (0.001 mol, 0.04 g) and 2-(4-methoxybenzylidene) malononitrile (0.01 mol, 1.84 g) (**2a**) were heated with stirring on hot plate at 100℃ for 30 minutes, the reaction mixture is cooled at room temperature then washed several times with water followed by drying .The solid that formed was recrystallized from ethanol forming the compound 3a with yield 90 % (m.p.142-144℃).Elemental *Anal*. Calc. for $C_{20}H_{15}N_3O_2$ (329.35): *C, 72.94; H, 4.59; N, 12.76*. Found C, 72.55; H, 4.13; N, 12.41.IR: 1605 cm^{-1} (C=N) , 2189 cm^{-1} (CN),2956,3062cm^{-1}, (NH_2).

^{1}H-NMR (DMSO-d6, δ, ppm): 3.82(s,3H,OCH_3), 5.02 (s,1H,CH of pyran), 6.86-6.89 (d,2H,p-substituted phenyl), 7.11-7.16 (d,2H,p-substituted phenyl), 7.43-7.63 (d,2H,in quinoline), 7.57- 7.60 (m,3H,pyridine ring), 9.01(s, 2H, NH_2 exchanged by D2O).

Synthesis of 2-amino-4-(4-fluorophenyl)-4H-pyrano [3, 2-h] quinoline -3-carbonitrile (3b)

A mixture of 8-hydroxy quinoline (0.01 mol, 1.45 g); sodium hydroxide (0.001 mol, 0.04 g) and 2-(4-fluorobenzylidene) malononitrile (0.01 mol, 1.72 g) (2b) were heated with stirring on hot plate at 100 ℃ for 30 minutes, the reaction mixture is cooled at room temperature then washed several times with water followed by drying .The solid that formed was recrystallized from ethanol forming the compound 3a with yield 85%(m. p.287-289℃).Elemental *Anal*. Calc. for $C_{19}H_{12}FN_3O$ (317.10): *C, 71.92; H, 3.81; N, 13.24*; *F, 5.99*. Found C, 71.62; H, 3.44; N, 13.01; F, 5.52.IR: 1604 cm^{-1} (C=N) , 2191 cm^{-1} (CN),2923cm^{-1},3063,3079cm^{-1}, (NH_2).

^{1}H-NMR (DMSO-d6, δ, ppm):5.01 (s, 1H,CH of pyran), 6.96-7.07(d,2H,p-substituted phenyl), 7.22-7.28(d,2H,p-substituted phenyl), 7.44-7.64 (d,2H,in quinoline), 7.59- 7.67 (m,3H,pyridine ring), 8.94(s,2H,NH_2 exchanged by D_2O) .

Synthesis of 7-(4-methoxyphenyl)-7H-pyrimido [5', 4':5, 6] pyrano [3, 2-h] quinolin-8(11H)-one (4a)

A mixture of quinoline-3-carbonitrile derivative **3a** (0.01 mol, 3.3 g) and formic acid (10 ml) was heated at reflux for 10 hr and then left to cool. The white crystals product thus formed was filtered off and recrystallized from ethanol forming the compound **3a** with yield 80%(m.p.213-215℃).Elemental *Anal*. Calc. for $C_{21}H_{15}N_3O_3$(357.36): *C, , 70.58; H, 4.23; N, 11.76*. Found C, 70.21; H, 3.98; N, 11.29.IR: 1506 cm^{-1} (C=O) , 1604 cm^{-1} (C= N),3400 cm^{-1} (NH) and absence of CN signal.

^{1}H-NMR (DMSO-d6,δ,ppm): 3.30 (s,3H,OCH_3) ,6.91 (s,1H,CH of pyrimidine),6.86-6.92 (d,2H,p-substituted phenyl),7.12-7.18 (d,2H,p-substituted phenyl), 7.25 (s,1H,NH exchanged by D_2O), 7.43-7.63 (d,2H,in quinoline), 7.57- 7.60 (m,3H,pyridine ring).

Synthesis of 7-(4-fluorophenyl)-7H-pyrimido [5', 4':5, 6] pyrano [3, 2-h] quinolin-8(11H)-one (4b)

A mixture of quinoline-3-carbonitrile derivative **3b** (0.01 mol, 3.17 g) and formic acid (10 ml) was heated at reflux for 10 hr and then left to cool. The white crystals product thus formed was filtered off and recrystallized from ethanol forming the compound 4**b** with yield 80%(m. p.242-244℃).Elemental *Anal*. Calc. for $C_{20}H_{12}F N_3 O_2$ (345.33): C, 69.56; H, 3.50; N, 12.17; F, 5.50. Found C, 69.33; H, 3.01; N, 11.87; F, 5.02.IR: 1506 cm^{-1} (C=O), 1605cm^{-1} (C=N), 3391 cm^{-1} (NH) and absence of CN signal.

^{1}H-NMR (DMSO-d6, δ, ppm): 5.01 (s,1H,CH of pyran), 6.91 (s,1H, CH of pyrimidine) , 7.28-7.31 (d,2H,p-substituted phenyl), 7.07-7.11 (d,2H,p-substituted phenyl), 7.31- 7.38 (d,2H,in quinoline) ,7.40-7.51 (m,3H,pyridine ring), 8.13 (s,1H,NH exchanged by D_2O).

Synthesis of 7-(4-methoxyphenyl)-7H-pyrimido [5', 4':5, 6] pyrano [3, 2-h] quinolin-8-amine (5a)

Compound quinoline-3-carbonitrile derivative **3a** (0.01 mol, 3.3g) was added to a mixture of formamide (10 ml), formic acid (5mLl) and dimethyl formamide (5mL). The reaction mixture was heated at reflux for 10hr and then left to cool. The solid product was filtered off and recrystallized from isopropanol with yield 75% to produce **5a** (m.p.105-107℃).Elemental *Anal*. Calc. for $C_{21}H_{16}N_4O_2$ (356.38) : C, 70.77; H, 4.53; N,

15.72. Found C, 70.42; H, 4.14; N, 15.32.IR: 1607 cm^{-1} (C=N), 2920cm^{-1}, 3010, 3062 cm^{-1} (NH_2) and absence of CN signal.

^{1}H-NMR (DMSO-d6, δ, ppm) : 3.86(s,3H,OCH_3) , 5.10 (s,1H,CH of pyran),6.80-6.83 (d,2H,p-substituted phenyl), 6.97 (s,1H, CH of pyrimidine),7.00-7.08 (d,2H,p-substituted phenyl), 7.25-7.28 (d,2H,phenyl protons), 7.36- 7.49 (m,3H,pyridine ring) , 8.82(s,2H,NH_2 exchanged by D_2O).

Synthesis of 7-(4-fluorophenyl)-7H-pyrimido [5', 4':5, 6] pyrano [3, 2-h] quinolin-8-amine (5b)

Compound quinoline-3-carbonitrile derivative **3b** (0.01 mol, 3.17 g) was added to a mixture of formamide (10 ml), formic acid (5mLl) and dimethyl formamide (5mL). The reaction mixture was heated at reflux for 10hr and then left to cool. The solid product was filtered off and recrystallized from isopropanol with yield 75% to produce **5b** (m. p.123-125°C).Elemental *Anal*. Calc. for $C_{20}H_{13}FN_4O$ (344.34)*: C, 69.76; H, 3.81; N, 16.27*; *F, 5.52*. Found C, 69.44; H, 3.67; N, 16.17; F, 5.22. IR: 1615 cm^{-1} (C=N), 2921cm^{-1},2998, 3064 cm^{-1} (NH_2) and absence of CN signal.

^{1}H-NMR (DMSO-d6, δ, ppm):5.05 (s,1H,CH of pyran), 6.99 (s,1H, CH of pyrimidine),7.02-7.07 (d,2H,p-substituted phenyl), 7.24-7.28 (d,2H,p-substituted phenyl), 7.30-7.38 (d,2H, in quinoline),7.40-7.50(m,3H,pyridine ring), 8.83(s,2H,NH_2 exchanged by D_2O).

Synthesis of 8-amino-7-(4-methoxyphenyl)-7H-pyrido [3', 2':5, 6] pyrano [3, 2-h] quinolin-10-ol (6a)

A mixture of quinoline-3-carbonitrile derivative **3a** (0.01 mol, 3.3g) and triethylorthoformate (1.7ml, 0.01 mole) in acetic anhydride (15 ml) was refluxed for 2hr. The mixture was dissolved and then precipitated, cooled, filtered off and dried. The solid product was crystallized from isopropanol to produce **6a** with yield 75%(m.p.242-244°C).Elemental *Anal*. Calc. for $C_{22}H_{17}N_3O_3$ (371.39) : *C, , 71.15; H, 4.61; N, 11.31*. Found C, 70.85; H, 4. 43.61; N, 11.01.IR: 1613 cm^{-1} (C=N),2841 cm^{-1},2926 ,3060 cm^{-1} (NH_2), 3383 cm^{-1} (OH) and absence of CN signal .

^{1}H-NMR (DMSO-d6, δ, ppm):3.72(s,3H,OCH_3) , 5.17 (s,1H,CH of pyran),6.86-6.92 (d,2H,p-substituted phenyl), 7.08-7.17 (d,2H,p-substituted phenyl), 7.20-7.31 (d,2H, in quinoline), 7.31- 7.34 (m,3H, pyridine ring) , 7.52 (s,1H, CH of pyridine) 8.89(s,2H,NH_2 exchanged by D_2O) , 10.47 (s,1H,OH exchanged by D_2O)

Synthesis of 8-amino-7-(4-fluorophenyl)-7H-pyrido [3', 2':5, 6] pyrano [3, 2-h] quinolin-10-ol (6b)

A mixture of quinoline-3-carbonitrile derivative **3b** (0.01 mol, 3.17 g) and triethylorthoformate (1.7ml, 0.01 mole) in acetic anhydride (15 ml) was refluxed for 2hr. The mixture was dissolved and then precipitated, cooled, filtered off and dried. The solid product was crystallized from isopropanol to produce **6b** with yield 80% (m. p.245-247°C).Elemental *Anal*. Calc. for $C_{21}H_{14}FN_3O_2$ (359.35)*: C, 70.19; H, 3.93; N, 11.69*; *F, 5.29*. Found C, 69.79; H, 3.68; N, 11.67; F, 4.97.IR: 1596 cm^{-1} (C=N),2924cm^{-1},3000,3063 cm^{-1} (NH_2), 3392 cm^{-1} (OH) and absence of CN signal .

^{1}H-NMR (DMSO-d6, δ, ppm):5.17 (s,1H,CH of pyran),7.03-7.07 (d,2H,p-substituted phenyl), 7.09-7.13 (d,2H,p-substituted phenyl),7.26-7.30(d,2H, in quinoline), 7.31- 7.34 (m,3H, Qu. pyridine ring),7.49 (s , 1H CH of pyridine), 9.82 (s,2H,NH_2 exchanged by D_2O) , 12.00(s,1H,OHexchanged by D_2O).

Reactions of thiosemicarbazide derivatives with halogenated reagents

General procedure for Synthesis of compounds 7a; 7b; 8a&8b:

1-(3-cyano-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinolin-2-yl)-3-phenylthiourea (7a)
1-(3-cyano-4-(4-fluorophenyl)-4H-pyrano [3, 2-h] quinolin-2-yl)-3-phenylthiourea (7b)
1-(3-cyano-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinolin-2-yl)-3-ethylthiourea (8a)
1-(3-cyano-4-(4-fluorophenyl)-4H-pyrano [3, 2-h] quinolin-2-yl)-3-ethylthiourea (8b)

To a suspension of quinoline-3-carbonitrile derivative 3a, b (0.01 mol) in dioxane (20 ml), the appropriate isothiocyanate (phenyl or ethyl) (0.01 mol) was added. The reaction mixture was heated at 80°C

with stirring for 2 hr and left over night at room temperature. The solid so obtained was filtered off and crystallized from the isopropanol to give 7a,b in good yields(80%,85%) or crystallized from ethanol to give 8a,b in good yields(85%,80%).

7ayield (80%)(m.p.123-125℃).Elemental *Anal*. Calc. for $C_{27}H_{20}N_4O_2S$ (464.54): *C, 69.81; H, 4.34; N, 12.06; S, 6.90*. Found C, 69.69; H, 4.01; N, 11.89; S, 6.67.IR: 1310 cm^{-1} (C=S) , 1614 cm^{-1} (C=N), 2190 cm^{-1} (CN), 3493 cm^{-1} (NH).

^{1}H-NMR (DMSO-d6, δ, ppm):3.71(s,3H,OCH_3) , 4.89 (s,1H,CH of pyran),6.86-6.89 (d,2H,p-substituted phenyl), 7.09-7.18 (d,2H,p-substituted phenyl), 7.21-7.27 (d,2H, in quinoline), 7.57-7.59 (m, 5H, phenyl),7.60-7.65 (m,3H,pyridine ring) , 8.34(s,1H,NH exchanged by D_2O) , 8.94(s,1H,NH exchanged by D_2O)

7byield (85%)(m.p.113-115℃).Elemental *Anal*. Calc. for $C_{26}H_{17}FN_4OS$ (452.50):*C,69.01;H,3.79;N,12.38;S* ,7.09;*F, 4.20* . Found C, 68.85; H, 3.51; N, 11.98; S, 6.79; F, 3.80.IR: 1310 cm^{-1} (C=S) , 1600 cm^{-1} (C=N), 2202 cm^{-1} (CN), 3370 cm^{-1} (NH).

^{1}H-NMR (DMSO-d6, δ, ppm):5.02 (s,1H,CH of pyran), 6.82-6.90 (d,2H,p-substituted phenyl), 7.09-7.18 (d,2H,p-substituted phenyl), 7.21-7.27 (d,2H,in quinoline), 7.57-7.59 (m, 5H, phenyl),8.25- 8.31 (m,3H,pyridine ring) , 8.80(s,1H,NHexchanged by D_2O) , 9.78(s,1H,NH exchanged by D_2O).

8a yield (85%) (m.p. 212- 214℃) .Elemental *Anal*. Calc. for $C_{23}H_{20}N_4O_2S$ (416.50): *C,* 66.33*; H,* 4.84*; N,* 13.45; *S,* 7.70. Found C, 65.96; H, 4.41; N, 13.07; S, 7.43.IR: 1375 cm^{-1} (C=S) , 1608cm^{-1} (C=N), 2189 cm^{-1} (CN), 3374 cm^{-1} (NH).

^{1}H-NMR (DMSO-d6, δ, ppm): 1.13 (t,3H,CH_3),3.71(s,3H,OCH_3),4.35 (q,2H,CH_2)4.92 (s,1H,CH of pyran) ,6.91-6.98 (d,2H,*p*-substituted phenyl), 7.04-7.07 (d,2H,p-substituted phenyl), 7.15-7.36 (d,2H,in quinoline),7.51- 7.54 (m,3H,pyridine ring) , 8.29(s,1H,NH exchanged by D_2O) ,8.84 (s,1H,NHexchanged by D_2O).

8b yield(80%) (m.p. 253-255℃).Elemental *Anal*. Calc. for $C_{22}H_{17}FN_4OS$ (404.46) :*C,65.33;H,4.24;N,13.85;S* ,7.93;*F,4.70* . Found C, 64.96; H, 3.94; N, 13.61; S, 7. 77; F, 4.48.IR: 1373cm^{-1} (C=S), 1601cm^{-1} (C=N), 2200 cm^{-1} (CN), 3370 cm^{-1} (NH).

^{1}H-NMR (DMSO-d6, δ, ppm):1.13 (t,3H,CH_3),4.35 (q,2H,CH_2) 4.92 (s,1H,CH of pyran),6.81-6.88 (d,2H,p-substituted phenyl), 7.04-7.07 (d,2H,p-substituted phenyl), 7.15-7.36 (d,2H, in quinoline), 7.51- 7.54 (m,3H,pyridine ring) ,8.29(s,1H,NH exchanged by D_2O),8.84 (s,1H, NH exchanged by D_2O).

General procedure for Synthesis of compounds 9a; 9b; 10a&10b:

(Z)-2-((4-hydroxy-3-phenylthiazol-2(3H)-ylidene) amino)-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (9a)
(Z)-4-(4-fluorophenyl)-2-((4-hydroxy-3-phenylthiazol-2(3H)-ylidene) amino)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (9b)
(Z)-2-((3-ethyl-4-hydroxythiazol-2(3H)-ylidene) amino)-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (10a)
(Z)-2-((3-ethyl-4-hydroxythiazol-2(3H)-ylidene) amino)-4-(4-fluorophenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (10b)

A mixture of phenylthiourea 7a,7b derivatives or ethylthiourea 8a,8b derivatives (0.02 mol) and ethylbromoacetate(0.022 mol, 2.4 ml) in absolute ethanol (25 ml) in presence of anhydrous sodium acetate (0.04 mole, 3.28 g) was refluxed for 4 hrs. The reaction mixture diluted with water after cooling and allowed to stand overnight, the solid that obtained was filtered off, dried and crystallized from ethanol for 9a,9b&10a or isopropanol in case of 10b in good yields(75% -85%)

9a yield(80%) (m.p 178-180℃).Elemental *Anal*. Calc. for $C_{29}H_{20}N_4O_3S$ (504.56): *C, 69.03; H, 4.00; N, 11.10;S* ,6.36. Found C, 68.78; H, 3.64; N,10.76;S,5.98.IR: 3429 cm^{-1} (OH), 1590cm^{-1} (C=N),2193cm^{-1}, (CN) and absence of CS signal .

9b yield (85%) (m.p.263-265℃).Elemental *Anal*. Calc. for $C_{28}H_{17}FN_4O_2$ (492.52): *C, 68.28; H, 3.48; N, 11.38; S, 6.51*; *F, 3.86*. Found C, 67.95; H, 3.12; N, 10.97; S, 6.23; F, 3.42 ^{1}H-NMR (DMSO-d6, δ, ppm):4.97 (s,1H of CH of pyran), 5.30 (s,1H,CH of thiazol), 5.50 (s,1H,CH of thiazol),6.81-6.92 (d,2H,p-substituted phenyl), 7.04-7.09 (d,2H,p-substituted phenyl), 7.20-7.37 (m, 5H, phenyl), 8.30-8.32 (d,2H,in quinoline), 8.79- 8.94 (m,3H,pyridine ring) , 11.00 (s,1H of OH , exchanged by D_2O).

10a yield (75%) %) (m.p 206-208℃).Elemental *Anal*. Calc. for $C_{25}H_{20}N_4O_3S$ (456.52)*:C, 65.77; H, 4.42; N, 12.27;S* ,7.02. Found C, 65.41; H, 4.17; N, 11.98; S, 6.87.IR: 3425 cm^{-1} (OH), 1611cm^{-1} (C=N), 2191cm^{-1}, (CN) and absence of CS signal. ^{1}H-NMR (DMSO-d6, δ, ppm):1.22 (t,3H,CH_3), 3.77(s,3H,OCH_3) , 3.90 (q,2H,CH_2), 4.75(s,1H of CH of pyran), 5.40 (s,1H,CH of thiazol),6.87-6.98 (d,2H,p-substituted phenyl), 7.15-7.21 (d,2H,p-substituted phenyl), 7.32-7.61(d,2H, in quinoline), 8.40- 8.98 (m,3H,pyridine ring) , 10.50 (s,1H of OH , exchanged by D_2O).

10b yield (75%) (m.p.205-207℃).Elemental *Anal*. Calc. for $C_{24}H_{17}FN_4O_2$(444.48): *C, 64.85; H, 3.86; N, 12.60;S* ,7.21;*F*,4.27. Found C, 64.65; H, 3.51; N, 12.27; S, 7.17; F, 4.08.IR: 3423 cm^{-1} (OH), 1611cm^{-1} (C=N), 2202cm^{-1}, (CN) and absence of CS signal. ^{1}H-NMR (DMSO-d6, δ, ppm):1.22 (t,3H,CH_3),3.98 (q,2H,CH_2), 4.75(s,1H of CH of pyran), 5.40 (s,1H,CH of thiazol),6.89-6.94 (d,2H,p-substituted phenyl), 7.10-7.12 (d,2H,p-substituted phenyl), 7.65-7.77(d,2H, in quinoline), 8.38- 8.97 (m,3H,pyridine ring) , 10.50 (s,1H of OH , exchanged by D_2O).

General procedure for Synthesis of compounds 11a; 11b; 12a&12b:

(Z)-4-(4-methoxyphenyl)-2-((4-methyl-3-phenylthiazol-2(3H)-ylidene) amino)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (11a)
(Z)-4-(4-fluorophenyl)-2-((4-methyl-3-phenylthiazol-2(3H)-ylidene) amino)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (11b)
(Z)-2-((3-ethyl-4-methylthiazol-2(3H)-ylidene) amino)-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (12a)
(Z)-2-((3-ethyl-4-methylthiazol-2(3H)-ylidene) amino)-4-(4-methoxyphenyl)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (12b)

A mixture of phenylthiourea 7a,7b derivatives or ethylthiourea 8a,8b derivatives (0.02 mol) and chloroacetone(0.022 mol, 1.8ml) in absolute ethanol (25 ml) in presence of anhydrous sodium acetate (0.04 mole, 3.28 g) was refluxed for 4 hrs. The reaction mixture diluted with water after cooling and allowed to stand overnight, the solid that obtained was filtered off, dried and crystallized from isopropanol to produce 11a,b&12a,b in good yields(75% -80%)

11a yield (80%) (m.p.208-210℃).Elemental *Anal*. Calc. for $C_{30}H_{22}N_4O_2S$ (502.59): *C, 71.69; H, 4.41; N, 11.15; S* , 6.38. Found C, 71.52; H, 4.11; N, 10.89; S, 5.98.
^{1}H-NMR (DMSO-d6, δ, ppm): 1.32 (s, 3H, CH_3 thiazol),3.71(s,3H,OCH_3) , 4.92 (s,1H,CH of pyran), 5.15 (s,1H,CH of thiazol),6.91-6.98 (d,2H,p-substituted phenyl), 7.04-7.07 (d,2H,p-substituted phenyl), 7.15-7.36 (d,2H, in quinoline), 7.37-7.41 (m, 5H, phenyl),7.51- 7.54 (m,3H,pyridine ring).

11b yield(80%) (m.p.233-235℃).Elemental *Anal*. Calc. for $C_{29}H_{19}FN_4OS$ (490.55): *C, 71.00; H, 3.90; N, 11.42;S 11.42*;*F*,3.87. Found C, 70.87 ; H, 3.63; N, 11.02; S, 11.19; F, 3.71. ^{1}H-NMR (DMSO-d6, δ, ppm): 1.30 (s, 3H, CH_3 thiazol),4.72 (s,1H of CH of pyran), 5.05 (s,1H,CH of thiazol), 6.92-6.98 (d,2H,p-substituted phenyl), 7.04-7.09 (d,2H,p-substituted phenyl), 7.11-7.35 (m, 5H, phenyl), 7.46-7.47 (d,2H, in quinoline), 7.52- 7.54 (m,3H,pyridine ring).

12a yield(80%) (m.p.>300℃).Elemental *Anal*. Calc. for $C_{29}H_{19}FN_4OS$ (490.55) *:C, 71.00; H, 3.90; F, 3.87; N, 11.42; S, 6.54*. Found C, 70.85 ; H, 3.76; N, 12.01; S, 6.96; F, 3.99.IR: 1603 cm^{-1}(C=N) ,2192 cm^{-1}(CN) and absence of CS signal.^{1}H-NMR (DMSO-d6, δ, ppm):1.22 (s,3H, CH_3 thiazol), 4.72 (s,1H of CH of pyran), 5.05 (s,1H,CH of thiazol), 6.92-6.98 (d,2H,p-substituted phenyl), 7.04-7.09 (d,2H,p-substituted phenyl), 7.11-7.35 (m, 5H, phenyl), 7.46-7.47 (d,2H, in quinoline), 7.52- 7.54 (m,3H, pyridine ring).

12b yield (75%) (m.p.127-130℃).Elemental *Anal*. Calc. for $C_{26}H_{22}N_4O_2S$ (454.54): *C, 68.70; H, 4.88; N, 12.33; S* , 7.05. Found C, 68.51; H, 4.41; N, 12.07; S, 6.85.

1H-NMR (DMSO-d6, δ, ppm): 0.84(s,3H of CH_3 ethyl),1.22 (t,3H,CH_3 thiazol), 3.63(s,3H,OCH_3) , 3.85 (q,2H,CH_2ethyl), 4.71 (s,1H of CH of pyran), 5.20(s,1H,CH of thiazol),6.89-6.97 (d,2H,p-substituted phenyl), 6.97-7.11 (d,2H, in quinoline), 7.04-7.07 (d,2H,p-substituted phenyl), 7.32- 7.51 (m,3H,pyridine ring) .

4-(4-fluorophenyl)-2-((Z)-((E)-5-(furan-2-ylmethylene)-4-oxo-3-phenylthiazolidin-2-ylidene) amino)-4H-pyrano [3, 2-h] quinoline-3-carbonitrile (13)

A mixture of quinoline-3-carbonitrile derivative 9b (0.01 mol,4.93 g) and furan-2-carbaldehyde (0.01 mol,0.8 ml) were refluxed in presence of three drops of triethyl amine using absolute ethanol (25 ml) for three hrs. The reaction mixture was left over night at room temperature. The solid so obtained was filtered off and crystallized from the isopropanol to give 13 with yield (75%) (m.p.135 -138 °C). Elemental *Anal*. Calc. for $C_{33}H_{19}FN_4O_3S$ (570.59)): *C, 69.46; H, 3.36; N, 9.82* ;S,5.62;*F*,3.33. Found C, 69.18; H, 2.97; N, 9.41; S, 5.21; F, 3.16.

^{1}H-NMR (DMSO-d6, δ, ppm):4.98 (s,1H,CH of pyran), 6.66-6.78 (d,2H,p-substituted phenyl), 7.10-7.12 (d,2H,p-substituted phenyl), 7.28-7.37 (m, 5H, phenyl), 7.40-7.53 (m,3H of furan), 7.56 (s,1H olefin = CH), 7.85-7.88(d,2H,phenyl in quinoline),8.38- 8.97 (m,3H,pyridine ring).

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