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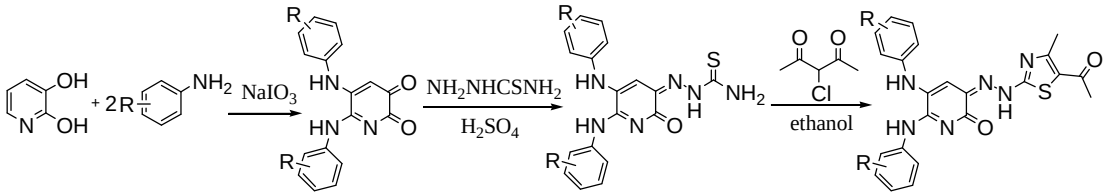

3b R= *o*-Cl, IC_{50} (μM) = **3.15** (MGC803)

IC_{50} (μM) = **8.17** (HCT116)

Design, synthesis and biological evaluations of novel pyridone-thiazole hybrid molecules as antitumor agents

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Abstract: A hybrid pharmacophore approach was adopted to design and synthesize new series of pyridone-thiazole hybrid compounds. The structures of the compounds were established by IR, ^{1}H NMR, ^{13}C NMR, and HRMS. All the newly prepared compounds (**3a-3m**) were *in vitro* evaluated for their antiproliferative activity against three human cancer cell lines, namely Colon cancer (HCT-116), gastric carcinoma (MGC803) and hepatocellular cancer (Huh7). Bioassay results demonstrated that most of the tested compounds showed potent anti-tumor activities against various cancer cells *in vitro*, and some compounds exhibited stronger effects than positive control 5-Fluorouracil (5-FU). Compound **3b** showed the best anti-tumor activity with IC_{50} values of 8.17 μM and 3.15 μM against HCT116 and MGC803 cell lines, respectively, which was 1.4-8.1 times more potent than 5-Fluorouracil (IC_{50} = 11.29 μM and 25.54 μM against HCT116 and MGC803 respectively). These findings suggest that compound **3b** may have potential to be developed as a promising lead for the design of novel anticancer small-molecule drugs.

Keywords: Pyridone Thiazole Hybrids Synthesis Antitumor activity

1. Introduction

Molecular hybridization is a valuable structural modification approach that comprises the incorporation of two or more pharmacophores into a single entity. In the last few years, hybrid drug design has emerged as a prime tool for the discovery of innovative anticancer therapies that can potentially overcome most of the pharmacokinetic drawbacks encountered when using conventional anticancer drugs.

The pyridin-2(*1H*)-one ring system present in various natural active substances [1,2]. The existence of a pyridin-2(*1H*)-one structural unit is a key to the pharmacological activities of many natural and synthetic

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drugs. A large number of compounds having pyridin-2(1*H*)-one ring system has been reported to possess different kind of biological activities like anti-tumor [3], cardiotonic [4], antituberculosis [5], antibacterial [6] and antihepatitis B virus [7]. Recently, pyridin-2(1*H*)-ones include 5-ethyl-1-phenyl-2-(1*H*)-pyridone [8], Camptothecin [9] and 3-(4,5,6,7-tetrahydro-3*H*-imidazo[4,5-*c*]pyridin-2-yl)-1*H*-quinolin-2-one [10] have been reported to show strong cytotoxicity against several human cancer cell lines.

Heterocycles containing the thiazole moiety are important targets in synthetic and medicinal chemistry because this fragment is a key moiety in a number of natural and synthetic biologically active agents [11,12]. In the last few decades, the clinical efficacy of tiazofurin and its analogs, and bleomycins (BLMs) has pointed out the importance of thiazole moiety for anticancer drug design. Bleomycins, glycopeptide antitumor antibiotics produced and isolated from *Streptomyces sp* have been clinically used to treat several types of cancers, like squamous cell carcinomas, malignant lymphomas and testicular cancers. In view of these reports and in continuation with the previous work, we therefore envisaged that integrating pyridin-2(1*H*)-one and thiazole moieties in one molecular platform could potentially produce novel compounds with significant synergistic antitumor properties (Fig. 1). These new pyridin-2(1*H*)-ones analogues bearing thiazole moiety (**3a-3m**) were prepared to verify their efficacy as potential anticancer agents.

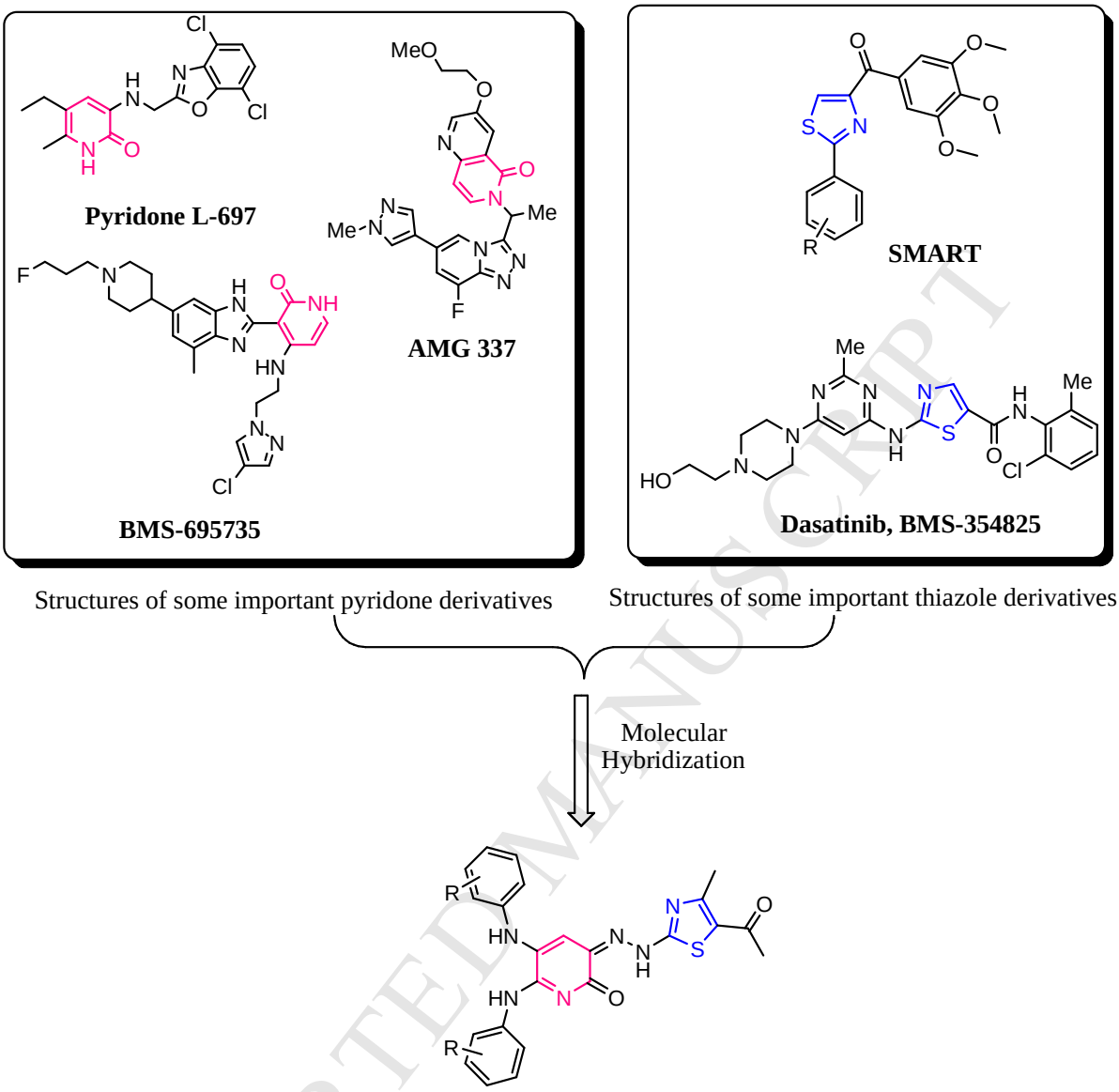


Fig. 1. Design of pyridone-thiazole hybrid compounds

2. Results and discussion

2.1. Chemistry

The synthetic pathways employed to prepare the new targeted derivatives are depicted in Scheme 1. The key intermediate **2** was prepared according to the procedure described in the literature [13]. First, 2,3-dihydroxypyridine was treated with appropriate aromatic amine in the presence of oxidant $NaIO_3$ in one pot via oxidation-Michael additions to yield 5,6-disubstituted pyridine-2,3-diones **1**, in the next step, 5,6-disubstituted pyridine-2,3-diones **1** was reacted with thiosemicarbazide through refluxing in anhydrous EtOH to afford the desired intermediate **2**. Subsequently, the intermediate **2** was reacted with

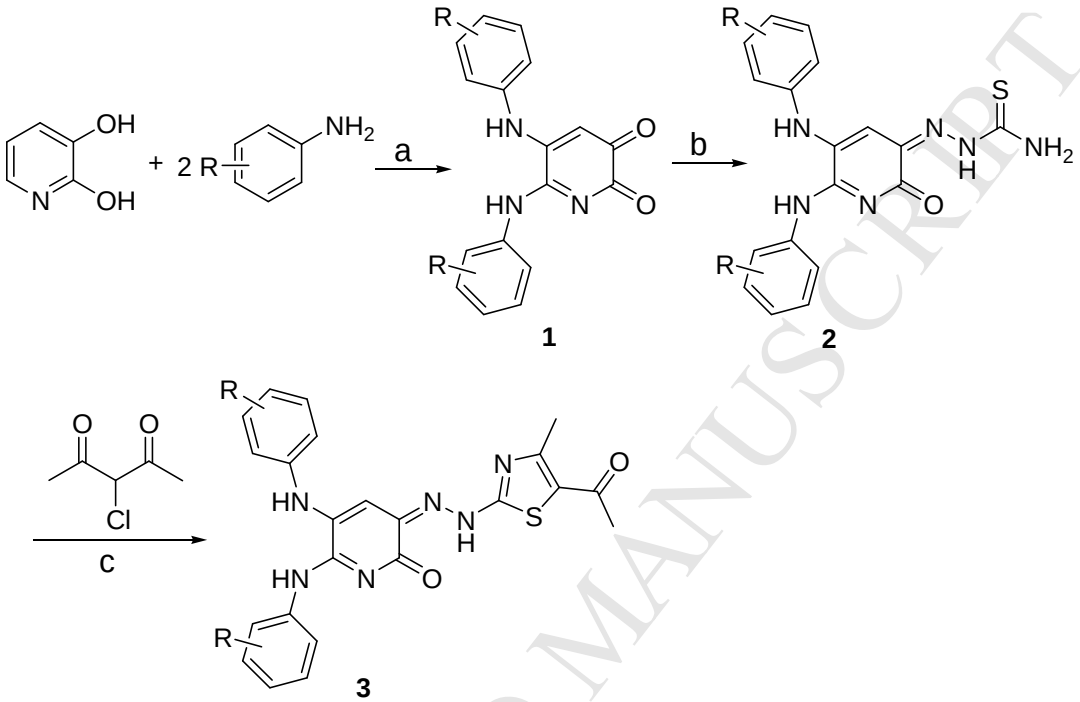
3-chloro-2,4-pentanedione via substitution-condensation reaction in refluxing ethanol to obtain the corresponding target compound **3**a-**3m** with good yields**.** The structures of all new synthesized compounds **3a-3m** were confirmed by ^{1}H NMR, ^{13}C NMR, IR and HRMS. IR spectra of the compounds **3** showed absorption bands due to the NH groups in the region 3140-3447 cm^{-1}, in addition to a carbonyl band in the region 1602-1657 cm^{-1}. In the ^{1}H NMR spectrum of **3a**, six singlet was observed (δ = 13.48, 7.98, 7.80, 6.68, 2.60, 2.47 ppm) for three NH group, pyridone-H, O=C-CH_3 and CH_3 respectively. The ^{13}C NMR spectrum of **3a** showed characteristic signals at δ 30.14 ppm (generated by the CH_3 of the acetyl group), 18.63 ppm (due to the CH_3 of thiazole), and 189.83, 167.85 ppm (arising from two carbonyl groups).

2.2. Biological activity

The *in vitro* antitumor activity of the newly synthesized compounds **3a-3m** was evaluated against a panel of three human cancer cell lines, including HCT116 (colon carcinoma cell), MGC803 (gastric carcinoma cell) and Huh7 (hepatoma carcinoma cell) by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. 5-Fluorouracil was used as positive control, and the results expressed as half-maximal inhibitory concentration (IC_{50}) values and are presented in Table 1, as mean values of experiments performed in triplicate. From the screening results in Table 1, it was observed that most of the synthesized compounds exhibited potent cytotoxic activities against the three human cancer cell lines. Special compounds **3a, 3b** and **3c** displayed higher cytotoxicity activity than 5-FU against HCT116 and MGC803 cell lines. The IC_{50} values of the most promising compound **3b** were 3.15 μM, and 8.17 μM against MGC803 and HCT116 cell lines, respectively, these values indicated that this compound was 8.1 and 1.4 times more active than 5-FU (IC_{50} values: 25.54 μM and 11.29 μM, respectively). Although most compounds showed potent antitumor activity, some compounds exhibited selectivity between the three human cancer cell lines. Such as compounds **3a, 3c** and **3j** showed the more potent inhibitory activity against HCT116 and MGC803 cell line and less inhibitory activity against Huh7 cell line.

From the antitumor activity against three human cancer cell lines, preliminary structure-activity relationships of the synthesized compounds were achieved. In general, compared with compounds **3a-3m**, substituent on benzene ring had great effect on the cytotoxic activity. Introduction of electron-withdrawing groups (such as Cl, Br and F) on the phenyl ring improved the activity. For example, the compound **3a, 3b, 3c, 3j, 3k** and **3l** displayed the more potent antitumor activity than the compound (**3m)** with no substituent on the phenyl ring against MGC803 and HCT116 cell lines**.** However, introduction of electron-donating

group (such as methyl and methoxyl group) on the phenyl ring reduced the inhibitory activity. For example, the compounds **3d, 3e, 3f, 3g, 3h** and **3i** displayed the lower antitumor activity than the compound (**3m)** against the three human cancer cell lines. These data also suggested that compound **3b** is a promising antitumor agent.



Scheme 1. Synthesis of the pyridone-thiazole hybrids derivatives (**3**). Reagent and conditions: (a) $NaIO_3$, rt; (b) $NH_2NHCSNH_2$, EtOH, H_2SO_4, reflux; (c) EtOH, heat to reflux

Table 1 *In vitro* antiproliferative activity of 3-(2-(4-acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-disubstituted pyridin-2(1*H*)-one (3)

Compound	R	IC_{50}[a] (μM)		
		HCT-116	MGC803[b]	Huh7[b]
3a	*m*-Cl	10.59±1.49	5.18±1.62	＞128
3b	*o*-Cl	8.17±1.89	3.15±1.68	21.47±1.97
3c	*p*-Cl	8.28±2.44	8.56±0.64	＞128
3d	*p*-CH_3	52.66±9.73	75.45±1.69	72.01±2.95
3e	*o*-CH_3	＞128	45.02±2.09	＞128
3f	*m*-CH_3	＞128	＞128	＞128
3g	*p*-CH_3O	＞128	82.64±1.77	＞128
3h	*o*-CH_3O	85.40±1.47	51.05±1.28	20.62±2.43
3i	*m*-CH_3O	＞128	＞128	＞128
3j	*m*-Br	22.17±1.28	21.56±1.33	＞128
3k	p-Br	13.21±1.26	9.12±1.45	17.87±1.86

3l	m-F	22.84±1.18	21.39±1.39	58.84±2.18
3m	H	25.34±5.93	13.54±1.16	19.05±1.69
5-FU		11.29±1.06	25.54±0.05	5.63±0.14

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control. Each value represents the mean ± S.E. of three experiments.

[b] HCT116 cells were the human colon carcinoma cells, MGC80 cells were the human gastric carcinoma cell and Huh7 cells were the human hepatocellular carcinoma cell.

3. Conclusions

In summary, in order to develop potent antitumor agents, we have designed and synthesized a series of novel hybrid molecules containing pyridone bearing thiazole moiety, and evaluated their *in vitro* antitumor activities against HCT116, MGC803 and Huh7 human tumor cell lines by MTT assay. Some of the compounds inhibited the proliferation better than positive control 5-Fluorouracil. In particular, compound **3b** showed the best inhibitory effect against MGC803 snd HCT116 cells, with IC_{50} value of 3.15±1.68 μM and 8.17±1.89 μM, respectively. Therefore, the results laid a foundation for further improving the potency and the selectivity of this series of compounds.

4. Experimental

4.1. General

Melting points were determined in open capillaries and were uncorrected. IR spectra were recorded on a PE-2000 spectrometer in KBr pellets and are reported in cm^{-1}. All NMR spectra were recorded on a Bruker AV-II 500 MHz NMR spectrometer, operating at 500 MHz for 1H, and 125 MHz for ^{13}C, TMS was used as an internal reference for ^{1}H and ^{13}C chemical shifts and $CDCl_3$ was used as solvent. Mass spectra were collected on a Waters Xevo Q-TOF HRMS instrument. 2,3-dihydroxypyridine was purchased from Aldrich; other commercially available materials were purchased from Aladdin-Reagent and Sinopharm Chemical Reagent, and were used without further purification.

4.2. Synthesis of 5,6-disubstituted pyridine-2,3-dione-3- thiosemicarbazone derivatives **(2)**

According to the literature method [13,14], sodium iodate (1.38g, 7.0 mmol) was added to a solution of 2,3-dihydroxypyridine (0.70g, 6.3 mmol) in 0.5 mol/L phosphate-buffered saline (35.0 mL, pH 6.0) and 125mL methanol. After stirring for 15 min, the appropriate aromatic amine (12.6 mmol) was added and the resulting mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, 150mL water was added, and the precipitate formed was isolated by

filtration. The resulting crude product was purified by column chromatography on silica gel to afford the corresponding **1.**

To a solution of 5,6-disubstituted pyridine-2,3-diones **1** (4 mmol) in anhydrous ethanol (60 mL) were added thiosemicarbazide (4.2 mmol) and sulfuric acid (0.04 mmol). The reaction mixture was refluxed and the reaction monitored by TLC**,** after the completion of the reaction, 200mL water was added, and the precipitate formed was isolated by filtration. The resulting crude product was purified by recrystallization with ethanol to afford the desired compounds **2.**

4.3. General procedure for the synthesis of 3-(2-(4-acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-disubstituted pyridin-2(1*H*)-ones **(3)**

Compound **2** (1 mmol) and 3-chloro-2,4-pentanedione (0.148 g, 1.1 mmol) were added to absolute ethanol (15 mL). The reaction mixture was heated at reflux for 12 h. After removal of solvent under reduced pressure, the residue was purified by silica gel chromatography using hexanes–ethyl acetate (7:3) to provide the desired compounds **3**.

4.3.1 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(3-chlorophenylamino) pyridin-2(1*H*)-one **3a**

Red solid; yield 71.8%; mp. 235-236 □; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.48 (s, 1H, NH), 7.98 (s, 1H, NH), 7.80 (s, 1H, NH), 7.53-7.46 (m, 3H, PhH), 7.37-7.31 (m, 2H, PhH), 7.15 (t, *J* = 7.5 Hz, 1H, PhH), 7.07 (t, *J* = 8.0 Hz, 1H, PhH), 6.98 (d, *J* = 7.5 Hz, 1H, PhH), 6.68 (s, 1H, pyridone-H), 2.60 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.83, 167.85, 158.86, 157.61, 142.28, 142.26, 136.31, 132.01, 130.86, 130.36, 130.27, 128.27, 127.76, 126.46, 126.19, 125.82, 125.69, 124.3, 121.91, 121.30, 104.64, 30.14, 18.63; IR(KBr) *v*: 3424, 3287, 3020, 2981, 1649, 1623, 1587, 1522, 1403, 1364, 1316, 1242, 1139, 1052 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}Cl_2N_6O_2S]^+$ $(M+H)^+$ 513.0662, found 513.0668.

4.3.2 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(2-chlorophenylamino) pyridin-2(1*H*)-one **3b**

Red powder; yield 67.0%; mp. 136-137 □; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.49 (s, 1H, NH), 7.95 (s, 1H, NH), 7.80 (s, 1H, NH), 7.54-7.46 (m, 3H, PhH), 7.38-7.31 (m, 2H, PhH), 7.16 (t, *J* = 7.5 Hz, 1H, PhH), 7.07 (t, 1H, *J* = 7.5 Hz, PhH), 6.98 (d, *J* = 8.0, Hz, 1H, PhH), 6.69 (s, 1H, pyridone-H), 2.61 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.79, 167.84, 158.83, 157.36, 142.27, 142.24,

136.30, 132.07, 130.86, 130.36(2C), 128.27, 127.76, 126.46, 126.22, 125.82, 125.67, 124.34, 121.91, 121.33, 104.60, 30.12, 18.57; IR(KBr) *v*: 3427, 3215, 3012, 2973, 1622, 1609, 1525, 1320, 1243, 1138, 1052 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}Cl_2N_6O_2S]^+$ $(M+H)^+$ 513.0662, found 513.0659.

4.3.3 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(4-chlorophenylamino) pyridin-2(1*H*)-one **3c**

Black powder; yield 55.0%; mp. 147-148 □; ^1H NMR (500 MHz, $CDCl_3$) *δ:* 13.45 (s, 1H, NH), 8.11 (s, 1H, NH), 7.41-7.36 (m, 4H, PhH), 7.33 (s, 1H, NH), 7.19 (d, *J* = 6.5 Hz, 2H, PhH), 6.88 (d, *J* = 7.0 Hz, 2H, PhH), 6.60 (s, 1H, pyridone-H), 2.60 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.81, 167.85, 158.86, 157.67, 143.57, 142.01, 137.80, 132.68, 131.07, 130.42, 130.40, 129.80, 129.15, 125.51, 122.71, 122.10, 103.86, 29.71, 18.59; IR(KBr) *v*: 3238, 3142, 3010. 2924, 1635, 1616, 1497, 1401, 1364, 1315, 1235, 1128, 970, 819, 637cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}Cl_2N_6O_2S]^+$ $(M+H)^+$ 513.0662, found 513.0663.

4.3.4 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(4-methylphenylamino) pyridin-2(1*H*)-one **3d**

Red solid; yield 62.0%; mp. 177-178 □; ^1H NMR (500 MHz, $CDCl_3$) *δ:* 13.44 (s, 1H, NH), 8.17 (s, 1H, NH), 7.33 (s, 1H, NH), 7.21 (t, *J* = 7.5 Hz,4H, PhH), 7.14 (d, *J* = 8.0 Hz, 2H, PhH), 6.82 (d, *J* = 8.0 Hz, 2H, PhH), 6.54 (s, 1H, pyridone-H), 2.59 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.36 (s, 6H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.80, 168.17, 159.17, 157.80, 142.57, 141.65, 136.55, 135.15, 133.94, 133.82, 131.13, 130.79, 130.20, 125.01, 121.99, 120.51, 102.28, 30.15, 29.71, 20.94, 18.63; IR(KBr) *v*: 3435, 3151, 3012, 2923, 1643, 1605, 1519, 1399, 1364, 1316, 1241, 1134 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_2S]^+$ $(M+H)^+$ 473.1754, found 473.1756.

4.3.5 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(2-methylphenylamino) pyridin-2(1*H*)-one **3e**

Purple powder; yield 68.0%; mp. 147-148 ℃; ^1H NMR (500 MHz, $CDCl_3$) *δ:* 13.45 (s, 1H, NH), 8.01 (s, 1H, NH), 7.38 (s, 1H, NH), 7.32-7.25 (m, 5H, PhH), 7.16-7.12 (m, 2H, PhH), 6.83 (d, *J* = 7.5 Hz, 1H, PhH), 6.32 (s, 1H, pyridone-H), 2.60 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.77, 168.11, 159.22, 157.74, 143.69, 141.20, 137.43, 134.01, 131.90, 131.60, 131.35, 131.11, 129.61, 127.45, 127.10, 125.56, 125.19, 125.03, 123.34, 119.52, 102.31,

30.14, 18.59, 17.82, 17.81; IR(KBr) *v*: 3445, 3310, 3020, 2931, 1657, 1627, 1519, 1459, 1401, 1363, 1326, 1221, 1123, 752cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_2S]^+$ $(M+H)^+$ 473.1754, found 473.1758.

4.3.6 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(3-methylphenylamino) pyridin-2(1*H*)-one **3f**

Red powder; yield 64.2%; mp. 242-243 □; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.48 (s, 1H, NH), 8.07 (s, 1H, NH), 7.37 (s, 1H, NH), 7.33-7.29 (m, 2H, PhH), 7.09-7.02 (m, 3H, PhH), 6.96 (d, *J* = 7.5 Hz, 1H, PhH), 6.74-6.72 (m, 2H, PhH), 6.64 (s, 1H, pyridone-H), 2.61 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.83, 168.12, 159.13, 157.75, 145.19, 141.61, 140.38, 139.69, 139.20, 133.31, 131.00, 130.11, 129.46, 126.28, 125.20, 124.92, 122.26, 121.09, 118.51, 117.51, 102.94, 30.16, 21.56, 21.49, 18.64; IR(KBr) *v*: 3363, 3165, 3018, 2923, 1647, 1612, 1530, 1401, 1365, 1328, 1130, 930, 640cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_2S]^+$ $(M+H)^+$ 473.1754, found 473.1757.

4.3.7 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(4-methoxyphenylamino) pyridin-2(1*H*)-one **3g**

Purple powder; yield 69.7%; mp. 202-203 □; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.44 (s, 1H, NH), 8.17 (s, 1H, NH), 7.21-7.19 (m, 3H, NH, PhH), 6.98-6.95 (m, 4H, PhH), 6.89 (d, *J* = 8.5 Hz, 2H, PhH), 6.38 (s, 1H, pyridone-H), 3.84 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 2.45 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.78, 168.19, 159.22, 157.82, 157.36, 156.81, 141.57, 138.02, 134.84, 131.91, 131.24, 124.94, 124.53, 121.88, 115.54, 114.91, 101.58, 55.60, 55.57, 30.14, 18.62; IR(KBr) *v*: 3361, 3198, 3013, 2925, 1638, 1602, 1512, 1399, 1364, 1290, 1241, 1137, 1031, 918, 777, 643cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_4S]^+$ $(M+H)^+$ 505.1653, found 505.1658.

4.3.8 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(2-methoxyphenylamino) pyridin-2(1*H*)-one **3h**

Purple powder; yield 62.0%; mp. 179-180 □; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.49 (s, 1H, NH), 8.14 (s, 1H, NH), 7.39 (s, 1H, NH), 7.35-7.29 (m, 2H, PhH), 6.85 (d, *J* = 8.0 Hz, 1H, PhH), 6.79-6.75 (m, 2H, PhH), 6.70-6.67 (m, 2H, PhH), 6.50 (d, *J* = 8.0 Hz, 1H, PhH), 6.48 (s, 1H, pyridone-H), 3.84 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.84, 168.09, 161.19, 160.72, 159.03, 157.72, 146.48, 141.83, 140.51, 132.88, 131.16, 130.80, 130.42, 125.22, 113.68, 112.52, 111.02, 109.09, 107.44, 106.56, 103.76, 55.43, 55.41, 30.15, 18.58; IR(KBr) *v*: 3322, 3195, 3015, 2925, 1654, 1621, 1528, 1401, 1364, 1238, 1140 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_4S]^+$ $(M+H)^+$ 505.1653, found 505.1656.

4.3.9 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(3-methoxyphenylamino) pyridin-2(1*H*)-one **3i**

Red powder; yield 72.0%; mp. 164-165 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.49 (s, 1H, NH), 8.13 (s, 1H, NH), 7.39 (s, 1H, NH), 7.36-7.30 (m, 2H, PhH), 6.87-6.75 (m, 4H, PhH), 6.70 (s, 1H, PhH), 6.50 (d, *J* = 8.0 Hz, 1H, PhH), 6.48 (s, 1H, pyridone-H), 3.85 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.85, 168.07, 161.18, 160.71, 159.04, 157.75, 146.49, 141.83, 140.51, 132.88, 131.15, 130.79, 130.42, 125.23, 113.67, 112.54, 111.01, 109.08, 107.43, 106.57, 103.76, 55.43, 55.41, 30.14, 18.59; IR(KBr) *v*: 3435, 3171, 3011, 2935, 1652, 1618, 1528, 1400, 1319, 1263, 1136, 1043, 694, 642cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{25}H_{25}N_6O_4S]^+$ $(M+H)^+$ 505.1653, found 505.1651.

4.3.10 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(3-bromophenylamino) pyridin-2(1*H*)-one **3j**

Red solid; yield 58.2%; mp. 136-137 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.49 (s, 1H, NH), 8.11 (s, 1H, NH), 7.39 (s, 1H, NH), 7.36-7.28 (m, 5H, PhH), 7.19 (d, *J* = 6.5 Hz, 1H, PhH), 7.11 (s, 1H, PhH), 6.86 (d, *J* = 7.5 Hz, 1H, PhH), 6.67 (s, 1H, pyridone-H), 2.61 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.86, 167.81, 158.86, 157.61, 146.54, 142.15, 140.71, 132.14, 131.55, 130.95, 130.12, 128.52, 126.89, 125.62, 124.06, 123.92, 123.82, 123.31, 119.59, 119.33, 104.68, 30.16, 18.56; IR(KBr) *v*: 3433, 3140, 3010, 2931,1634, 1615, 1586, 1522, 1400, 1366, 1319, 1238, 1138, 776, 637cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}Br_2N_6O_2S]^+$ $(M+H)^+$ 600.9651, found 600.9656.

4.3.11 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(4-bromophenylamino) pyridin-2(1*H*)-one **3k**

Red solid; yield 51.0%; mp. 154-155 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.51 (s, 1H, NH), 8.03 (s, 1H, NH), 7.58-7.52 (m, 4H, PhH), 7.32 (s, 1H, NH), 7.14 (d, *J* = 8.0 Hz, 2H, PhH), 6.82 (d, *J* = 7.5 Hz, 2H, PhH), 6.63 (s, 1H, pyridone-H), 2.62 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 189.95, 167.99, 158.99, 157.81, 143.71, 142.15, 137.93, 132.81, 131.21, 130.56, 130.40, 129.93, 129.29, 125.65, 122.85, 122.23, 103.99, 30.29, 18.72; IR (KBr) *v*: 3447, 3168, 3012, 2929, 1634, 1609, 1524, 1340, 1325, 1260, 1129 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}Br_2N_6O_2S]^+$ $(M+H)^+$ 600.9651, found. 600.9652.

4.3.12 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(3-fluorophenylamino) pyridin-2(1*H*)-one **3l**

Black powder; yield 65.5%; mp. 153-153 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) *δ:* 13.50 (s, 1H, NH), 8.05 (s, 1H, NH), 7.44-7.40 (m, 2H, NH, PhH), 7.36 (t, *J* = 7.5 Hz, 1H, PhH), 7.03-6.98 (m, 2H, PhH), 6.94 (t, *J* = 8.0 Hz, 1H, PhH), 6.83 (t, *J* = 8.0 Hz, 1H, PhH), 6.74 (s, 1H, PhH), 6.72-6.67 (m, 2H, PhH, pyridone-H),

2.62 (s, 3H, CH_3), 2.48 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 189.82, 167.79, 163.85 (d, J = 248.75 Hz), 163.52 (d, J = 245.00 Hz), 158.82, 157.68, 146.80 (d, J = 8.8 Hz), 142.09, 140.98 (d, J = 10.0 Hz), 132.05, 131.70 (d, J = 8.8 Hz), 130.94 (d, J = 8.8 Hz), 130.12, 125.54, 116.52 (d, J = 3.8 Hz), 116.20 (d, J = 2.5 Hz), 112.45 (d, J = 21.3 Hz), 110.58 (d, J = 21.3 Hz), 108.48 (d, J = 22.5 Hz), 107.97 (d, J = 25.0 Hz), 104.79, 30.17, 18.57; IR(KBr) v: 3446, 3158, 3015, 2926, 1638, 1609, 1499, 1401, 1363, 1316, 1228, 1115 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{19}F_2N_6O_2S]^+$ $(M+H)^+$ 481.1253, found 481.1251.

4.3.13 (*Z*)-3-(2-(4-Acetyl-5-methylthiazol-2-yl)hydrazono)-5,6-*bis*(phenylamino)pyridin-2(1*H*)-one **3m**
Brown powder; yield 56.2%; mp. 237-238 □; ^{1}H NMR (500 MHz, $CDCl_3$) δ*:* 13.47 (s, 1H, NH), 8.10 (s, 1H, NH), 7.46-7.40 (m, 5H, NH, PhH), 7.27 (d, J = 5.5 Hz, 2H, PhH), 7.22 (t, J = 7.5 Hz, 1H, PhH), 7.15 (t, J = 7.0 Hz, 1H, PhH), 6.94 (d, J = 7.5 Hz, 2H, PhH), 6.67 (s, 1H, pyridone-H), 2.61 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 189.80, 168.08, 159.08, 157.74, 145.20, 141.69, 139.28, 133.15, 130.85, 130.28, 129.69, 125.49, 125.22, 124.01, 121.47, 120.63, 103.13, 30.14, 18.61; IR(KBr) v: 3442, 3167, 3020, 2925, 1632, 1612, 1494, 1401, 1313, 1233, 1110 cm^{-1}; HRMS (ESI, *m/z*) calcd for $[C_{23}H_{21}N_6O_2S]^+$ $(M+H)^+$ 445.1441, found 445.1443.

4.4. Anti-proliferative assay

The anticancer activities of the prepared compounds against HCT-116, MGC803 and Huh7 cells were evaluated as described elsewhere with some modifications [15]. Target tumor cell lines were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. Cells were harvested during logarithmic growth phase and seeded in 96 well plates at a density of 2×10^4 cells/mL, and incubated at 37 □ in a 5% CO_2 incubator. After 24 h incubation at 37 □, 10 μl tested compounds was added to 96-well plates and cultured at 37 □ for 72 h, 20 μl of MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 □. Discarded the suspension and added 150 mL of dimethyl sulfoxide (DMSO) to each well and shook the plates to dissolve the dark blue crystals (formazan); the absorbance was measured using a microplate reader at a wavelength of 570 nm. Each concentration was analyzed in triplicate and the experiment was repeated three times. The average 50% inhibitory concentration (IC_{50}) was determined from the dose-response curves according to the inhibition ratio for each concentration. There was a good reproducibility between replicate wells with standard errors below 10%. The results were summarized in

Table 1.

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- ► We explored synthetic methods of novel pyridone-thiazole hybrid molecules
- ► All newly synthesized compounds were screened for their anticancer activities.
- ► Most of the prepared compounds exhibited cytotoxicity against various cancer cells *in vitro*. (3b: IC_{50} (μM) = 3.15 (MGC803); 8.17 (HCT116))
- ► The structure-activity relationship was discussed.