

## Anticancer activities of some synthesized 2,4,6-trisubstituted pyridine candidates.

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### Abstract

We herein report the anti-cancer activities of some synthesized pyridine derivatives substituted with thiazole, pyrazole and triazole moieties. A series of these derivatives 1-12 were synthesized and evaluated as anti-inflammatory, analgesic, anticonvulsant and antiparkinsonian agents before. Twelve compounds were conveniently screened for their *in vitro* cytotoxicity against a wide range of cell lines and they are also showed potent activities against renal and prostate cancer cell lines. The *in vivo* antirenal cancer and antiprostate cancer of the most active *in vitro* compounds was estimated and founded highly potent. In search for the mechanism of action of anticancer activities it was founded that these compounds exert its action via histone deacetylase inhibition and inhibition of p53 ubiquitination.

### Keyword:

2,4,6-Trisubstituted pyridine, Heterocyclic derivatives, Anti-cancer activities.

Accepted on February 25, 2016.

### Abbreviations:

ARF: Alternative Reading Frame; DMEM: Dulbecco's Modified Eagle's Medium; DMSO: Dimethyl Sulfoxide; HLI98: HDM2 Ligase Inhibitor 98 Class.

### Introduction

Thieno[2,3-b]pyridine candidates which substituted with pyridine, cyclopentyl, tetrahydroquinoline, pyrimidine, 1,6-naphthiridin, benzofuro[2,3-b]pyridine, imidazo[1,2-c]pyrimidine, triazolo[1,5-a]pyrimidine were synthesized and used a new agents for their *in vitro* antitumor activities against liver HepG-2 and breast MCF-7 cell lines [1-3]. Many pyridine derivatives were synthesised and founded to have cytotoxic activities *in vitro* against a wide range of cell lines especially renal and prostate types [4]. Imidazopyridine derivatives have potent antitumor activities [5]. In addition, two series of 4,6-diaryl-2-imino-1,2-dihydropyridine-3-carbonitriles and imidazo[2,1-b]pyridine/ pyrimidine chalcones were also synthesized and evaluated for their *in vitro* capacity to inhibit PDE3A and the growth of the human HT-29 colon adenocarcinoma tumor cell line [6-8]. In view of these observations and in continuation of our previous work [9-16] in biological and pharmacological studies for heterocyclic

candidates, we herein reported the anti-cancer activities of some heterocyclic substituted pyridine derivatives.

### Experimental

#### Chemistry

All the tested compounds 1-12 were confirmed by physical and spectroscopic evidences according to the previously reported procedure [16].

#### Cytotoxic activities

*In vitro* determination of IC<sub>50</sub> of tested compounds against different cancer cell line via using the MTT Assay

The cytotoxicity of the newly synthesized compounds against cancer cell lines *in vitro* was performed with the MTT assay according to the previous reported method [17].

#### *In vivo* determination of the cytotoxic activities

Effects of compounds tested compounds on prostate cancer *in vivo*

To investigate the effect of tested compounds on prostate cancer cells *in vivo* use the adopted methods [18,19].

**In vivo antirenal cancer**

**Cell lines:** OUR-10 cells were maintained in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (FCS). OUR-10 and DU145 cells were maintained in Dulbecco's modified Eagle's medium containing 10% heat-inactivated FCS.

**Proliferation assay:** The experimental method which was used in proliferation assay has been adopted from Oka et al. [20].

**Xenograft model:** The experimental method which was used in xenograft model has been adopted from Liang et al. [21].

**In vitro inhibition of histone deacetylase:** The experimental method which was used *in vitro* inhibition of histone deacetylase has been adopted from Yoshid et al., [22] and Farooq et al., [23].

**Biological assay in vitro ubiquitination assay:** The experimental method which was used for biological assay *in vitro* ubiquitination assay has been adopted from Roxburgh et al., [24].

**Non-fluorescent in vitro ubiquitination assays:** The experimental method which was used for non-fluorescent *in vitro* ubiquitination assays has been adopted from Roxburgh et al., [24].

**Statistical analysis**

The data were expressed as means plus; standard deviation (s.d.). Statistical analysis was performed by Student's t-test (two-tailed). The criterion for statistical significance was taken as P<0.05.

**Results and Discussion**

In continuation of our previous work, a series of substituted pyridine, pyrazole, triazole and thiazolotriazole derivatives 1-12 (Figure 1) were synthesized before and screened as anti-inflammatory, analgesic, anticonvulsant and antiparkinsonian agents [16]. Herein, we report the activities of these compounds for evaluation as anticancer agents.

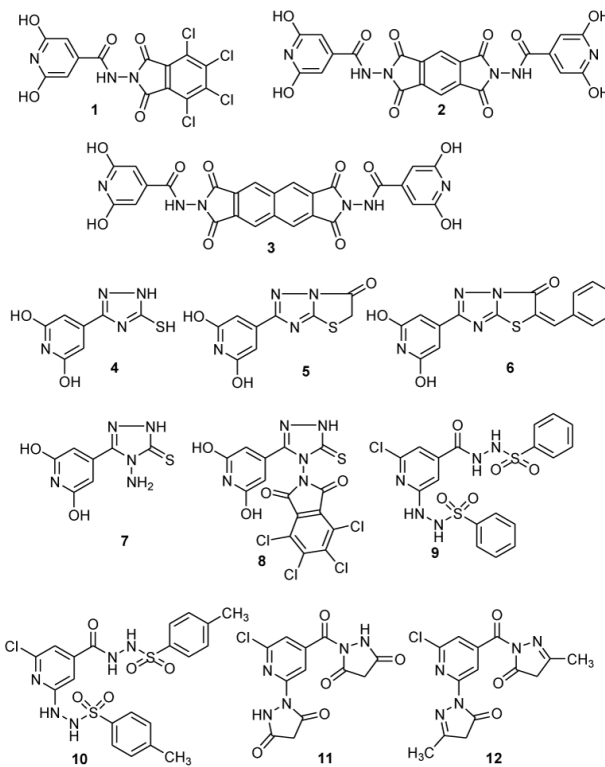


Figure 1. Chemical structure for tested compounds 1-12.

**Anti-cancer activities**

The cytotoxicity of the newly synthesized compounds against cancer cell lines *in vitro* was performed with the MTT assay according to the previous reported [17].

All the tested compounds showed potent cytotoxic activities *in vitro* against aKB6, SKOV-3, SF-268, NCI H 460, RKOP27, PC3, OUR-10, HL60, U937, K561, G361, SK-MEL-28, GOTO, NB-1, HeLa, MCF-7, HT1080 and HepG2 at micro molar level for all cell except for PC3, OUR-10 the potent activities lies on nanomolar level (Table 1).

Table 1. In vitro cytotoxicity activities of the tested compounds 1-12 against several cancer cell lines using MTT assay.

Comp. No.	IC50 μ M Tumor cell growth inhibition						
	KB6	SK OV-3	SF-268	NCI H 460	RKOP27	PC3	OUR-10
1	0.29	0.98	0.88	0.067	0.03	0.00057	0.000099
2	0.28	0.97	0.87	0.066	0.044	0.00055	0.000091
3	0.27	0.96	0.86	0.055	0.055	0.00053	0.000086
4	0.26	0.88	0.85	0.054	0.046	0.00051	0.000084
5	0.25	0.86	0.74	0.053	0.063	0.00049	0.000079
6	0.24	0.84	0.67	0.052	0.052	0.00048	0.000073
7	0.23	0.83	0.58	0.047	0.043	0.00046	0.000068

8	0.21		0.76		0.48	0.038	0.054	0.00043	0.000066		
9	0.14		0.56		0.36	0.026	0.022	0.00034	0.000052		
10	0.16		0.67		0.38	0.037	0.013	0.00035	0.000054		
11	0.13		0.55		0.35	0.024	0.033	0.00023	0.000044		
12	0.12		0.54		0.33	0.021	0.032	0.00021	0.000043		
Comp. No.	Leukemia			Melanoma		Neuro-blastoma		Cervical	Breast	Fibrosa-rcoma	liver
	HL60	U937	K561	G361	SK-MEL-28	GOTO	NB-1	HeLa	MCF-7	HT1080	Hep-G2
1	0.88	0.87	0.35	0.56	0.67	0.56	0.45	0.34	0.45	0.56	0.23
2	0.77	0.95	0.46	0.67	0.76	0.65	0.42	0.25	0.46	0.34	0.67
3	0.64	0.06	0.57	0.75	0.84	0.73	0.57	0.13	0.55	0.12	0.45
4	0.55	0.95	0.65	0.84	0.93	0.47	0.43	0.96	0.64	0.32	0.43
5	0.63	0.84	0.74	0.93	0.82	0.46	0.67	0.87	0.75	0.45	0.75
6	0.34	0.73	0.85	0.82	0.76	0.68	0.85	0.78	0.86	0.32	0.98
7	0.47	0.52	0.76	0.76	0.65	0.59	0.77	0.59	0.75	0.68	0.78
8	0.56	0.66	0.67	0.67	0.58	0.44	0.68	0.67	0.66	0.45	0.66
9	0.26	0.48	0.37	0.54	0.29	0.16	0.59	0.55	0.34	0.67	0.24
10	0.47	0.37	0.28	0.45	0.47	0.35	0.48	0.36	0.25	0.63	0.57
11	0.35	0.27	0.46	0.35	0.30	0.26	0.30	0.44	0.43	0.54	0.43
12	0.24	0.35	0.55	0.26	0.19	0.37	0.29	0.35	0.14	0.24	0.12

**Table 2.** *In vivo* antiprostata carcinoma for tested compounds 1-12.

Comp. No.	% Decrease in PC3 tumor volume
Control	230
1	53
2	46
3	45
4	42
5	38
6	36
7	31
8	29
9	27
10	28
11	25
12	22

### ***In vivo* antiprostata cancer**

The *in vivo* anti-prostate cancer activities of the tested compounds were estimated and culminated on the tested compounds exerts potent anti-prostate carcinoma in the

following descending order 12, 11, 9, 10, 8, 7, 6, 5, 4, 3, 2 and 1 (Table 2).

### ***In vivo* anti-renal cancer**

In this study, from table 3 compounds 1-12 were used to test the effect on renal cancer cell lines. As shown in table 3, treatment with tested compounds reduced cell viability of renal cancer cells (OUR-10) in a time-dependent manner [18].

### ***Xenograft model***

We examined whether tested compounds could inhibit the growth of OUR-10 tumors in mice. We selected an injection dose of 7  $\mu$ M/Kg 3 times per week because this dose effectively inhibited *in vivo* OUR-10 tumor growth without adverse effects in a preliminary study. About 6 weeks after the start of treatment with compounds 12, 11, 9, 10, 8, 7, 6, 5, 4, 3, 2 and 1, the increase in OUR-10 tumor volume was measured and given in table 4. The *in vivo* antirenal cancer activities of the tested compounds were estimated and culminated on the tested compounds exerts potent anti prostate carcinoma in the following descending order 12, 11, 9, 10, 8, 7, 6, 5, 4, 3, 2 and 1.

### ***Mechanism of anticancer activities***

In search for the mechanism of action of the anticancer activities of the tested compounds the author's resorts to many

*in vitro* assays for many targeted enzymes and cancer modulation molecules, these experiments revealed on the following two mechanistic pathways.

**Table 3.** Cytotoxic effect of treatment with tested compounds 1-12 against OUR-10.

Comp. No.	%Cell Viability after time for cell line OUR-10		
	Day 1	3 Days	5 Days
1	38	25	12
2	34	22	11
3	31	21	10
4	29	19	9
5	28	17	8
6	27	16	8
7	25	15	7
8	24	13	7
9	18	10	6
10	22	11	6
11	14	9	5
12	12	8	5

**Table 4.** *In vivo* antirenal carcinoma for tested compounds 1-12.

Comp. No.	Tumor volume mm3
<b>Control</b>	<b>988</b>
1	291
2	288
3	278
4	255
5	243
6	238
7	224
8	212
9	167
10	181
11	155
12	138

***In vitro* Inhibition of histone deacetylase**

All the tested compounds inhibited the enzyme histone decarboxylase in the following descending order 12, 11, 9, 10, 8, 7, 6, 5, 4, 3, 2 and 1 (Table 5).

***Ubiquitination assay***

All the testes compounds showed inhibition of p53 ubiquitination, the most active ones were 12, 11, 9, 10, 8, 7, 6, 5, 4, 3, 2 and 1 (Table 6) [18].

**Table 5.** IC50 of histone decarboxylate inhibitor activities of the tested compounds 1-12.

Comp. No.	IC50 (nM)
1	0.0066
2	0.0059
3	0.0058
4	0.0057
5	0.0056
6	0.0055
7	0.0054
8	0.0047
9	0.0045
10	0.0046
11	0.0044
12	0.0043

**Table 6.** IC50 of p53 ubiquitination of the newly synthesized compounds 1-12 (*in vitro*).

Comp. No.	IC50 of p53 ubiquitination
1	0.77
2	0.71
3	0.69
4	0.67
5	0.57
6	0.55
7	0.53
8	0.44
9	0.48
10	0.42
11	0.33
12	0.31
Diph. Imidazole	0.26 (17)

**Conclusion**

All tested compounds inhibited *in vivo* prostate and renal carcinoma via inhibition of the histone decarboxylase and inhibition of p53 ubiquitination also inhibited the EGFR and VEGFR-2 kinase.

## Structural Activity Relationships

- Chloropyridine and pyrazoles essential for potent activities but the methyl pyrazole exerts more potent activities than the oxo-ones, while sulfones showed the least potent activities.
- Hydroxy pyridines provide less potent activities than the chloropyridines.
- Fusion of another heterocyclic ring system into the triazole ring greatly reduces the activities.
- Macrocylic moieties and polychloro ones greatly reduces the activities.

## Acknowledgement

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group project no. RGP -172.

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