Synthesis and *in vitro* antitumor activity of novel naphthyridinone derivativesXue-Dong Jia^{a, b}, Shuo Wang^a, Ming-Hua Wang^a, Ming-Liang Liu^{a, *}, Gui-Min Xia^a, Xiu-Jun Liu^a, Yun Chai^a, Hong-Wei He^{a, *}^a Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China^b The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China

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ABSTRACT

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A series of naphthyridinone derivatives based on **1a** (a precursor of Voreloxin) were designed and synthesized. Seven compounds having > 70% inhibition against HL60 at 30 $\mu\text{mol/L}$ were further evaluated for their *in vitro* antitumor activity by SRB assay. Results reveal that thiazol-2-yl and 3-aminomethyl-4-benzoyloxyimino-3-methylpyrrolidin-1-yl groups are optimal at the N-1 and C-7 positions of naphthyridinone core, respectively. **10j** exhibits broad-spectrum activity (IC_{50} : < 0.5-6.25 $\mu\text{mol/L}$) against all of the tested cell lines including Etoposide- and/or **1a**-resistant ones, and is 1.3 -fold to >100 -fold more potent than the two references against eight of these cell lines.

1. Introduction

Cancers still remain the first and second leading causes of death in economically developed and developing countries, respectively [1,2]. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within the next 2 decades [3]. Encouragingly, substantial progress has been made to develop more effective and safer strategies for treating cancers, and many classes of antitumor agents with diverse novel structures have been introduced in the market or under development in the past decade [4]. However, there continues to be a need for new antitumor drugs from either novel scaffolds or further structural modifications of existing drugs.

Quinolones represent an extremely successful family of antibiotics that have a broad-spectrum antibacterial activity and relatively few side effects [5]. Among of them, ciprofloxacin, ofloxacin and levofloxacin are also used as second-line drugs to treat tuberculosis [6]. Recently, quinolones as "privileged building blocks" have been reported to display many "nonclassical" biological profiles, such as antitumor, anti-HIV-1 integrase, anti-HCV-NS3 helicase and -NS5B-polymerase activities [7,8]. It's exciting that Voreloxin (Fig. 1) with a broad-spectrum antitumor activity [9] the first quinolone antitumor drug, was approved as an orphan drug for the treatment of acute myeloid leukemia by the US FDA in 2009, and phase I-III clinical trials in patients with various tumors are currently ongoing [10].

Fig. 1. Chemical structures of Voreloxin and **1a**

In the course of our search for more potent antitumor agents, we focused our interest on structural modifications of Voreloxin. Given that 3-aminopyrrolidine, but not (3S, 4S)-3-methoxy-4-(methylamino)pyrrolidine, is commercially available, compound **1a** (Fig. 1) possessing the *in vitro* antitumor activity comparable to Voreloxin [11] was chosen as the lead compound in our work. First, the thiazol-2-yl group at the N-1 position of **1a** was replaced with its isosteres (1*H*-imidazol-2-yl, oxazol-2-yl, etc.) to examine whether it is the optimal group of this position. On the other hand, our previous works have emphasized the importance of an oxime functional moiety of the C-7 side chain with respect to biological activities of quinolones [12-15]. Thus, a four, five- or six-membered nitrogen

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heterocycle containing an alkoxyimino group was introduced at the C-7 position instead of the 3-aminopyrrolidin-1-yl one of **1a**, to identify if a nitrogen heterocycle with an oxime moiety is permitted at this position. Our primary objective was to optimize the potency of these compounds against human cancer cell lines. A simple structure-activity relationship (SAR) study was also explored to facilitate the further development of the naphthyridinone derivatives.

1. Experimental

2.1 Synthetic chemistry

To study the effect of various substituents at the N-1 position of the 7-(3-aminopyrrolidin-1-yl)naphthyridinone core, eight analogues of **1a** were designed. A synthetic route to these compounds is illustrated in Scheme 1. The key intermediates **6a-i** were readily prepared from 2, 6-dichloronicotinic acid (**2**) via a five-step procedure [14,16]. Hydrolysis of the core esters **6a-d** in HCl-HOAc followed by condensation with 3-*N*-Boc-aminopyrrolidine in the presence of triethylamine gave **8a-d**. However, the esters **6e-i** were not converted to the corresponding acids in a similar manner as for preparation of **8a-d**. After various attempts, **8e-i** were successfully obtained from **6e-i** by condensation with the side chain compounds and then hydrolysis of the resulted esters **7e-i**. The Boc-protecting group of **8a-i** was removed by hydrogen chloride gas in dichloromethane to yield the desired naphthyridinone derivatives **1a-i** as hydrochloric acid salts.

Scheme 1. Synthesis of 7-(3-aminopyrrolidin-1-yl)naphthyridinone derivatives **1a-i**.

The synthetic route of novel 1-(thiazol-2-yl)naphthyridinones **10a-n** containing nitrogen heterocycles with an alkoxyimino group was depicted in Scheme 2. Condensation of **6a** with various side chain compounds[12,17-19] and then hydrolysis of the resulted esters **9a-n** yielded **10a-n**.

Scheme 2. Synthesis of 1-(thiazol-2-yl)naphthyridinone derivatives **10a-n**.

Since the oxime group can exist in the *E* or *Z* configuration, it was necessary to determine the geometries of all the oxime target compounds **10a-n**. Preparing X-ray quality single crystals of any oxime intermediate or product met with no success in this study, but the oxime geometry would be expected to have the *E*-configuration according to the data in published papers [20,21]. The concrete synthetic procedures, the physical characteristics, and ¹H NMR for all the synthesized compounds are listed in the Supporting information.

2.2 Anti-tumor activities

All the synthesized target compounds were preliminarily investigated for their *in vitro* activity against HL60 (leukemia) at the concentration of 30 μM by SRB (Sulforhodamine B) assay [22]. And the compounds having >70% inhibition were subjected to IC₅₀ (50 % inhibition concentrations) determination against ten human cancer cell lines, including HL60, HepG2 (liver carcinoma), HCT-116 (colon cancer), A549 (lung adenocarcinoma), PANC-1 (pancreatic carcinoma), Hela (cervical cancer), DU145 (prostatic cancer), SKOV3 (ovarian carcinoma), MCF-7 (breast cancer) and MCF-7/DOX (Doxorubicin-resistant MCF-7) by SRB assay.

2. Results and discussion

The inhibitory effects of the 7-(3-Aminopyrrolidin-1-yl) naphthyridinone derivatives **1a-i** and 1-(thiazol-2-yl)naphthyridinone derivatives **10a-n** were evaluated and listed in Table 1 and Table 2. Unfortunately, contrary to our predictions, the results indicated that all the newly synthesized 7-(3-Aminopyrrolidin-1-yl) naphthyridinone derivatives **1b-i** (inhibition rates: 0-28%) were much less active than the reference **1a** (75%, Table 1). Therefore, our modifications are not effective, and the thiazol-2-yl group is optimal for the N-1 position.

The IC₅₀ values were compared with those of Etoposide and **1a** (Table 2). The selected 1-(thiazol-2-yl)naphthyridinones have potent activity against these tested human cancer cell lines. All of them (IC₅₀: <0.5-49.23 μmol/L) are more active or comparable to Etoposide and **1a** (IC₅₀: 1.17->50 μmol/L) against HCT-116, A549, SKOV3 and MCF-7/DOX. Contrary to **1a**, compounds **10h-j** show significantly better activity against MCF-7/DOX (IC₅₀: <0.5 μmol/L) than MCF-7 (IC₅₀: 5.57-31.04 μmol/L). Moreover, **10j** was found to have a broad-spectrum activity (IC₅₀: <0.5-6.25 μmol/L) against all of the tested cell lines including Etoposide- and/or **1a** - resistant ones, and is 1.3 ->100 fold more potent than those of the two references against these cell lines, except HL60 and HepG2.

According to the biological evaluation results shown in Tables 1 and 2, the antitumor activity of the naphthyridinone derivatives in this study depends on both of the groups at the N-1 and C-7 positions. The relative contribution of the heteroaromatic ring at the N-1 position to activity is as follows: thiazol-2-yl (**1a**) >> 1*H*-imidazol-2-yl (**1f**) > thiophen-3-yl (**1d**), and the others (**1b**, **1c**, **1e**, **1g-i**) have no activity (Table 1).

On the other hand, 1-(thiazol-2-yl)naphthyridinones **10a-n** generally exhibit *in vitro* activity (Table 1), which indicates that a nitrogen heterocycle with an oxime group is permitted at the C-7 position. Although a simple SAR is difficult to explore, the sizes of the heterocycle and the oxime group are important for the activity. For example, the activity imparted to the 7-(3-aminomethyl-3-methylpyrrolidin-1-yl)naphthyridinone ring by the alkyl group of the oxime moiety is as follows: benzyl > ethyl > methyl (**10j** vs **10i** vs **10h**), which suggests that simply increasing the lipophilicity could improve the activity (Table 2). Above all, thiazol-2-yl and 3-aminomethyl-4-benzyl oxyimino-3-methylpyrrolidin-1-yl groups are the most active at the N-1 and C-7 positions of naphthyridinone core, respectively.

Table 1
Structures and *in vitro* activity of compounds **1a-i** and **10a-n** against HL60 (at 30 μmol/L)

Compd.	R or X	% inhibition	Compd.	R or X	% inhibition	Compd.	R or X	% inhibition
1a		75	1i		0	10h		75
1b		0	10a		58	10i		77

1c		0	10b		56	10j		80
1d		10	10c		73	10k		85
1e		0	10d		9	10l		54
1f		28	10e		78	10m		83
1g		0	10f		44	10n		39
1h		0	10g		32			

Table 2
In vitro activity of selected compounds against ten cell lines

compd	IC ₅₀ ^a (μmol/L)									
	HL60	HepG2	HCT-116	A549	PANC-1	Hela	DU145	SKOV3	MCF-7	MCF-7/DOX
10c	1.62	16.73	39.40	17.27	> 50	> 50	> 50	49.24	35.82	16.54
10e	0.77	13.24	27.69	11.62	> 50	32.52	> 50	28.80	24.78	23.56
10h	12.10	15.72	23.32	22.93	> 50	42.08	26.50	22.68	31.05	< 0.5
10i	6.20	10.85	21.15	8.74	> 50	21.72	15.41	26.49	17.28	< 0.5
10j	1.18	2.99	5.62	4.05	5.30	6.25	5.52	5.18	5.57	< 0.5
10k	2.76	13.59	29.70	16.00	> 50	44.62	> 50	25.65	19.63	1.43
10m	4.80	6.77	12.68	5.19	> 50	15.76	> 50	34.29	12.21	26.42
1a	1.17	3.17	18.74	22.06	> 50	41.64	6.96	33.90	14.59	29.21
etoposide	2.91	1.97	> 50	48.05	> 50	> 50	18.05	44.97	16.27	> 50

^a IC₅₀ values were presented as the concentration of drug inhibition 50% cell growth and determined by at least three separate tests and reported

3. Conclusion

In summary, structural modifications of compound **1a** (a precursor of Voreloxin) at the N-1 position (various heteroaromatic rings instead of the thiazol-2-yl) and C-7 position (four-/five-/six-membered nitrogen heterocyclic amine moieties with an oxime group instead of 3-aminopyrrolidin-1-yl one), respectively, were made in this study. Our results reveal that thiazol-2-yl and 3-aminomethyl-4-benzyloxyimino-3-methylpyrrolidin-1-yl groups are optimal at the N-1 and C-7 positions of naphthyridinone core, respectively. The most active compound **10j** shows considerable broad-spectrum antitumor activity (IC₅₀: <0.5-6.25 μmol/L) against all of the tested cell lines including Etoposide- and/or **1a**-resistant ones and is 1.3- >100 fold more potent than those of the two references against these cell lines, except HL60 and HepG2.

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Graphical Abstract

Synthesis and *in vitro* antitumor activity of novel naphthyridinone derivatives

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