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Synthesis of novel strobilurin–pyrimidine derivatives and their antiproliferative activity against human cancer cell lines

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ABSTRACT

A series of new strobilurin–pyrimidine analogs were designed and synthesized based on the structures of our previously discovered antiproliferative compounds **I** and **II**. Biological evaluation with two human cancer cell lines (A549 and HL60) showed that most of these compounds possessed moderate to potent antiproliferative activity. Two potent candidates (**8f**, IC_{50} = 2.2 nM and **11d**, IC_{50} = 3.4 nM) were identified with nanomolar activity against leukemia cancer cell line HL60 for further development. This activity represents a 1000- to 2500-fold improvement compared to the parent compounds **I** and **II** and is 20- to 30-fold better than the chemotherapy drug, doxorubicin. The present work provides strong incentive for further development of these strobilurin–pyrimidine analogs as potential antitumor agents for the treatment of leukemia.

Strobilurins, isolated from specific fungi, constitute a large family of compounds that possess a broad spectrum of fungicidal activity with low toxicity towards mammalian cells and environmentally benign characteristics.[1,2] Strobilurin derivatives are also used as agrochemical,[1–6] antiviral,[7] anti-malarial,[8] and anti-microbial agents.[9] In particular, the strobilurin–pyrimidine moiety has been extensively utilized as a drug scaffold in medicinal chemistry, and at present, three commercialized fungicide products, including azoxystrobin, fluoxastrobin, and fluacrypyrim, incorporate this substructure (Fig. 1).[1,2,5,6] In addition, it has been found that strobilurin–pyrimidine analogs have antitumor activity through inhibition of STAT3 activation.[10] For example, fluacrypyrim (Fig. 1) inhibited leukemia cancer cell growth by predominantly G1 arrest with significant decreases of cyclin D1 protein and mRNA levels.[10]

Our research group has been investigating the potential of strobilurin–pyrimidine derivatives for antitumor applications. Previously, we discovered two analogs **I** and **II** (Fig. 2), which possess good antiproliferative activity against lung cancer (**I**, IC_{50} = 3.4 μM, **II**, IC_{50} = 3.0 μM, A549) and leukemia (**I**, IC_{50} = 5.5 μM, **II**, IC_{50} = 3.3 μM, HL60) cell lines.[11] As a continuation of our previous work,[11–15] herein we report a follow-up lead optimization to improve the potency, and also structure–activity relationship (SAR) investigation results.

Our structural exploration strategy for the hit compounds (**I** and **II**) is illustrated in Figure 3. For compound **I**, first, the 5-*n*-butyl group was fused with the 6-methyl group on the pyrimidine substructure to form a 5- or six-member carbocycle (**8a**, **8e**) to restrict the possible conformations. Second, a series of halogens and/or alkyl groups (**8b–d**, **8f–g**) was introduced to the 2-phenylamino moiety to study substituent effects on SAR. Similarly, for compound **II**, the phenylamino group was replaced with substituted anilines (**9a–f**) or aliphatic amines (**10a–g**). Next, incorporation of a methyl group at the 5-position of the pyrimidine gave the analog **11** series. Furthermore, replacement of the toxophore (β-methoxyacrylate, Q^1) moiety with groups Q^2–Q^5 afforded derivatives **12–15**.

Key building blocks for the construction of strobilurin–pyrimidine analogs **8–15** were the 2-aminopyrimidin-4-ols **4–7**, which were generally synthesized via condensation of guanidines with the corresponding β-keto esters (Scheme 1). Guanidines **1** and **2** were readily prepared according to our previously reported methods.[14,15] Most of the β-keto esters were purchased from commercial sources, and intermediate **3** was prepared by methylation of ethyl 4,4,4-trifluoro-3-oxobutanoate with methyl bromide.[16] Benzyl halides (**III–V**, Schemes 2 and 3) were prepared according to literature procedures.[17–20] Nucleophilic substitution of the benzyl halides with phenols **4–7** in the presence of potassium carbonate as base afforded the target molecules **8–13** in 65–85% yields (Schemes 2 and 3). The structure of compound **9b** was further confirmed by X-ray crystallography (see 'Supplementary data'). Treatment of

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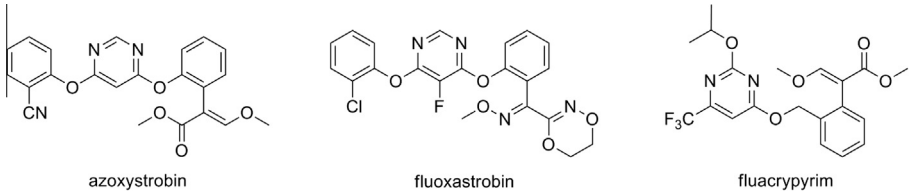


Figure 1. Structure of representative strobilurin–pyrimidine scaffolds.

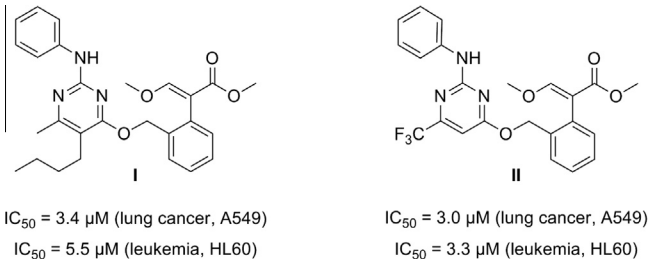


Figure 2. Structures of antiproliferative strobilurin–pyrimidine derivatives **I** and **II**.

compounds **12** and **13** with methylamine gave derivatives **14** and **15**, respectively, in good yields (Scheme 3).

The in vitro antiproliferative activity of strobilurin–pyrimidine analogs against two human cancer cell lines (A549 and HL60) was evaluated using MTT or SRB assays according to Mosmann's methods.[21] IC_{50} (μM) values (concentration required to achieve 50% inhibition of tumor growth) of tested compounds are summarized in Tables 1 and 2.

Fusion of the 5-*n*-butyl and 6-methyl groups on the pyrimidine substructure as a 6-member ring (**8a**) did not improve the antiproliferative activity. However, elimination of one ring carbon gave the cyclopenta[*d*]pyrimidine analog (**8e**), which showed equally good or better activity against both cancer cell lines compared to compound **I**. Thus, building on the structure of **8e**, introduction of halogens (**8f–g**) to the aniline moiety was probed to improve potency. For example, the 2,4-dichloro-3-methyl analog (**8g**, IC_{50} = 0.2 μM) led to a 17-fold improvement of activity against the human lung cancer cell line A549 (vs **I**, IC_{50} = 3.4 μM). A breakthrough was the 2,3,4-trifluoro analog (**8f**), which possessed an IC_{50} of 2.2 nM against leukemia cancer cell line HL60. This activity represents an increased potency of 2500-fold better than **I** (IC_{50} = 5.5 μM) and 37-fold greater than doxorubicin (IC_{50} = 0.082 μM), a chemotherapy drug with broad-spectrum antitumor activity. Overall, in this series, substituent effects for the 5- and 6-positions of the pyrimidine ring could be ranked as

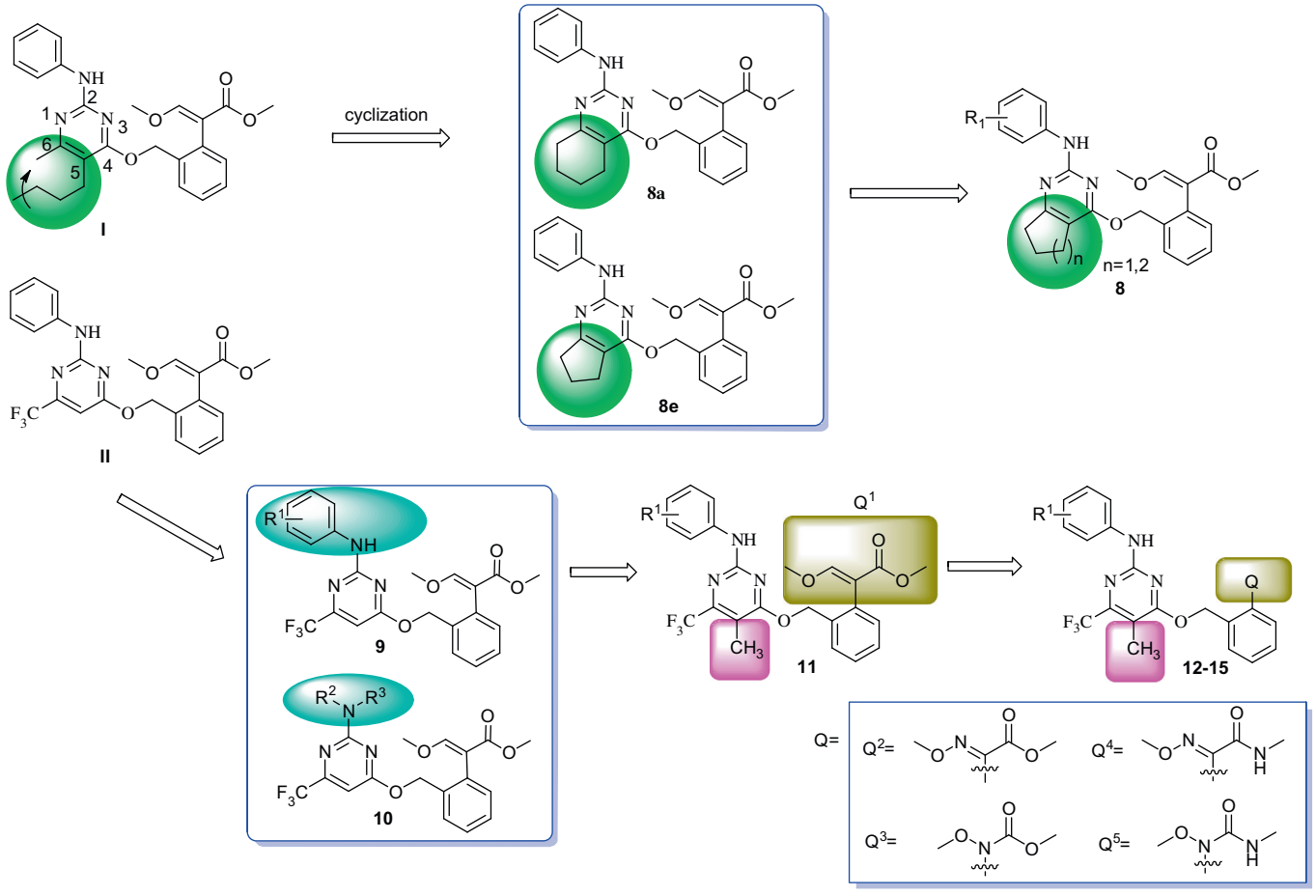
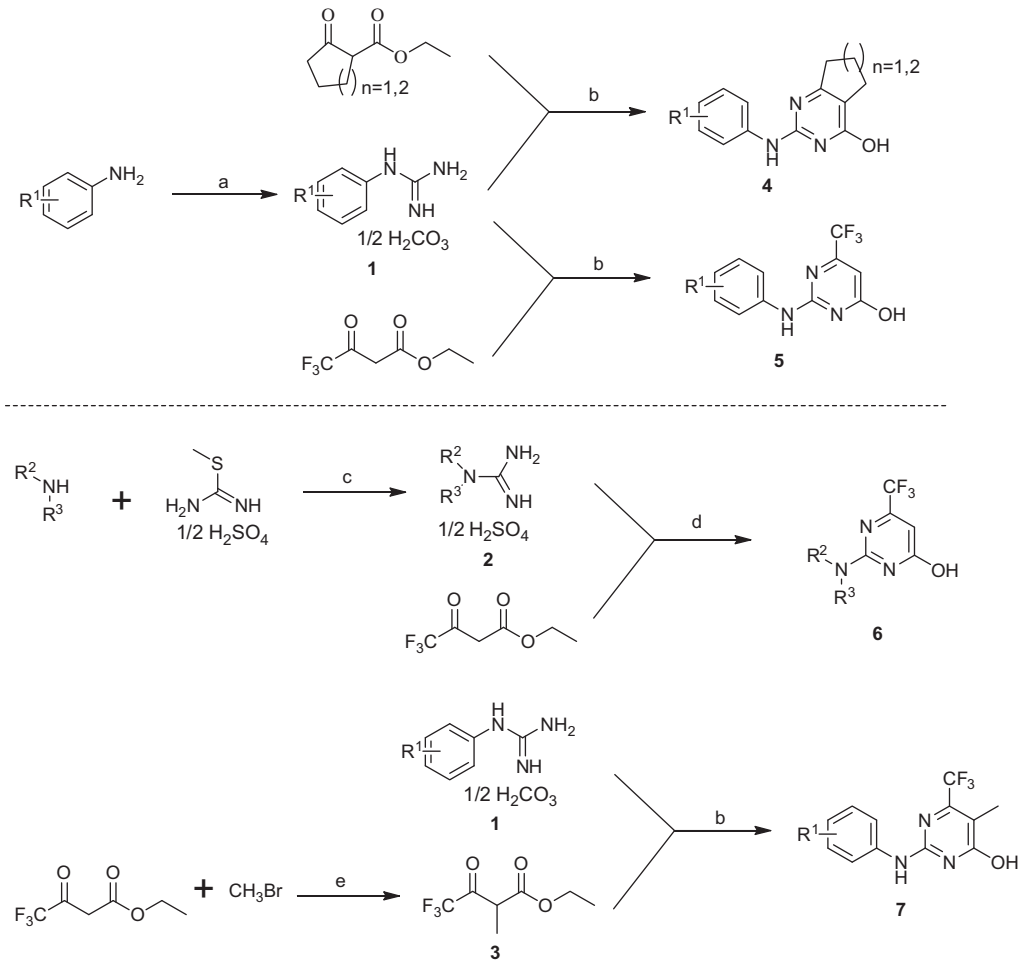
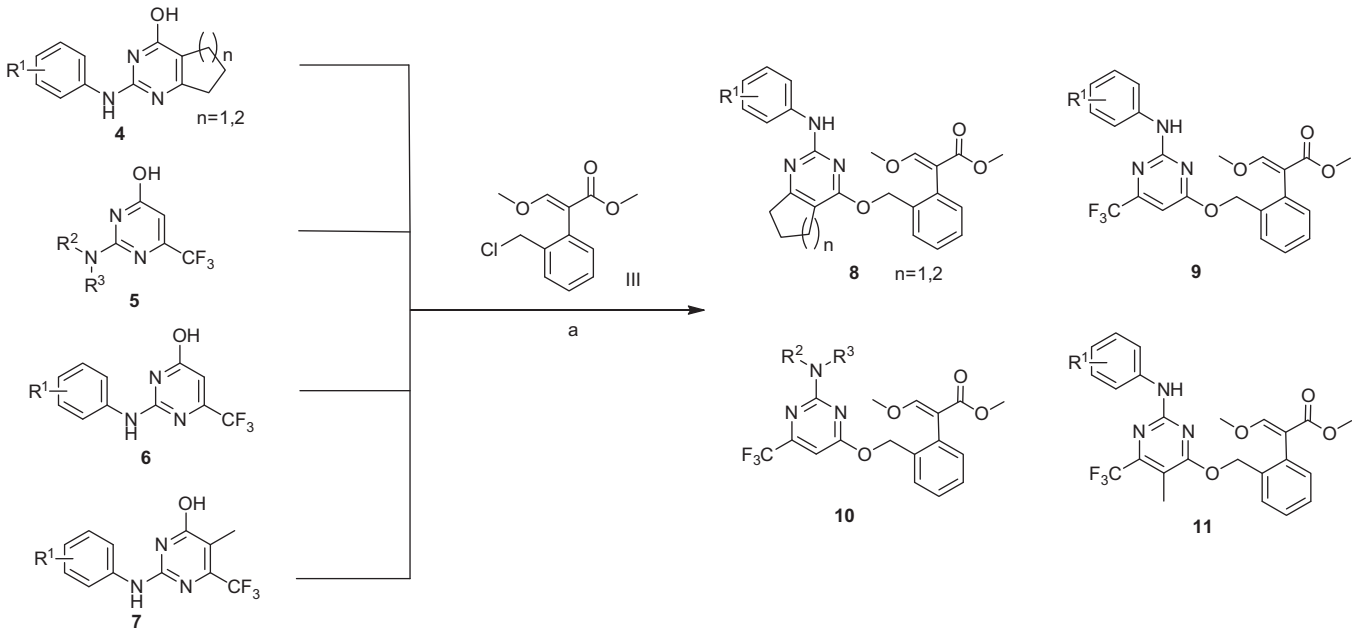


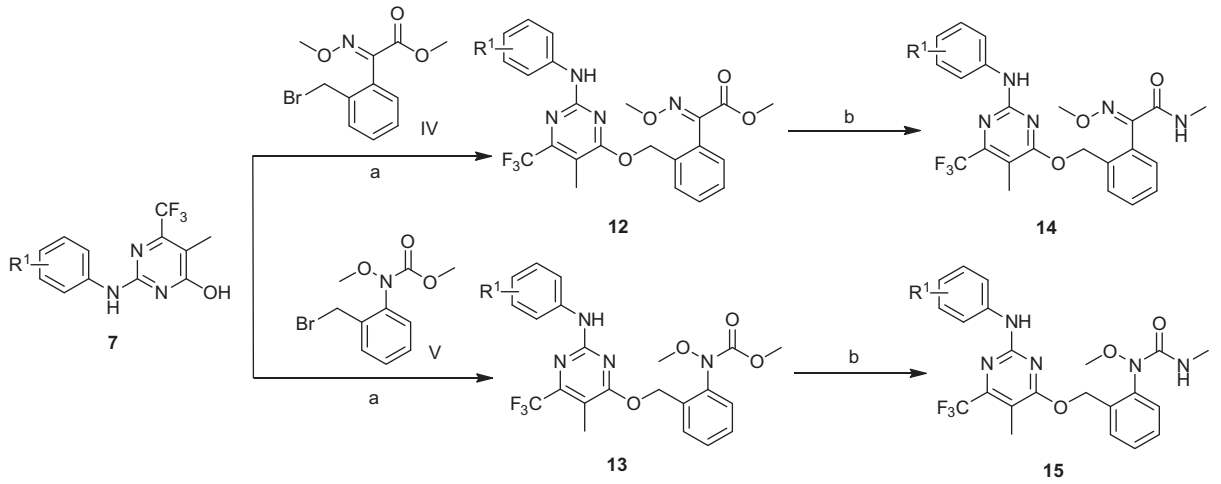
Figure 3. Structure exploration strategy based on compounds **I** and **II**.



Scheme 1. Synthesis of 2-aminopyrimidin-4-ols **4**–**7**. Reagents and conditions: (a) NH_2CN, concd. HCl, Na_2CO_3; (b) toluene, reflux; (c) H_2O; (d) $NaOCH_3$, CH_3OH; (e) NaH, toluene.

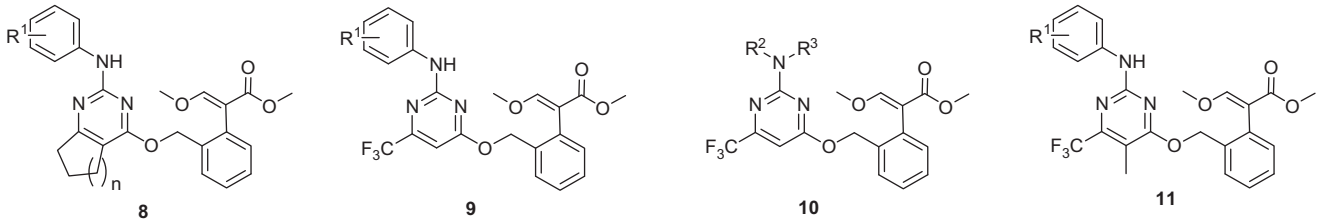


Scheme 2. Synthesis of strobilurin–pyrimidines **8**–**11**. Reagents and conditions: (a) K_2CO_3, DMF, 80 °C.



Scheme 3. Synthesis of strobilurin–pyrimidines **12**–**15**. Reagents and conditions: (a) K_2CO_3, DMF, 80 °C; (b) NH_2CH_3, CH_3OH.

Table 1
Structure–activity relationship study of compounds **8**–**11** in A549 and HL60 cell lines



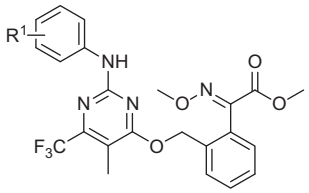
Compounds	R^1	*n*	R^2	R^3	IC_{50} (μM)	
					A549[a]	HL60[b]
I	—	—	—	—	3.4	5.5
8a	H	2	—	—	9.5	11.8
8b	2,3-Di-Cl	2	—	—	16.4	1.1
8c	2,4-Di-F	2	—	—	1.1	8.2
8d	2,3,4-Tri-F	2	—	—	25.6	2.3
8e	H	1	—	—	3.1	1.9
8f	2,3,4-Tri-F	1	—	—	1.0	0.0022
8g	2,4-Di-Cl-3-Me	1	—	—	0.2	7.7
II	—	—	—		3.0	3.3
9a	4-Cl	—	—	—	1.3	19.0
9b	2,4-Di-Cl	—	—	—	>100	>100
9c	2-F, 4-Cl	—	—	—	1.3	2.7
9d	2,4-Di-F	—	—	—	1.0	4.8
9e	2,6-Di-F	—	—	—	41.4	12.1
9f	2,3,4-Tri-F	—	—	—	1.0	13.6
10a	—	—	Me	H	93.7	76.7
10b	—	—	Et	H	3.2	0.3
10c	—	—	Me	Me	6.0	11.1
10d	—	—	Et	Et	17.9	15.0
10e	—	—	^{i}Pr	H	9.8	3.4
10f	—	—	Cyclopropyl	H	10	6.5
10g	—	—	$-(CH_2)_5-$		4.9	51.6
11a	H	—	—	—	7.4	0.7
11b	4-Cl	—	—	—	5.5	0.7
11c	2,4-Di-Cl	—	—	—	0.4	2.9
11d	2,4-Di-F	—	—	—	3.1	0.0034
11e	2,3,4-Tri-F	—	—	—	5.0	0.8
Fluacrypyrim		—	—	—	9.8	6.9
Doxorubicin		—	—	—	0.5	0.082

[a] IC_{50}s were measured using SRB assay.
[b] IC_{50}s were measured using MTT assay.

follows: 5-cyclic fused pyrimidine (**8e–g**) >5-*n*-butyl-6-methyl pyrimidine (**I**) >6-cyclic fused pyrimidine (**8a–d**).

According to the encouraging SAR results from the derivative **I** series, halogens were introduced into the aniline substructure of compound **II** to give analogs **9**. Most of these derivatives (**9a**, **9c–d** and **9f**) exhibited slightly improved activity against the human lung cancer cell line A549 compared to compound **II**. It is worth noting that introduction of chlorine (**9b**) to the 2-position instead

Table 2
Structure–activity relationship study of compounds **12–15** in A549 and HL60 cell lines



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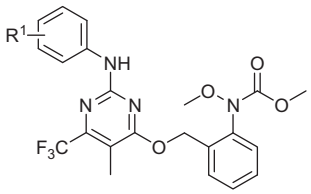

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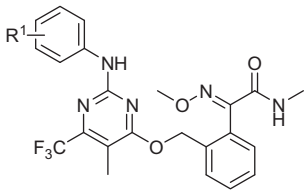

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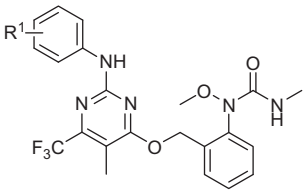

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Compounds	R^1	IC_{50} (μM)	
		A549[a]	HL60[b]
II	—	3.0	3.3
12a	2,4-Di-F	83.5	48.1
12b	2,3,4-Tri-F	82.1	50.5
13a	2,4-Di-F	>100	68.1
13b	2,3,4-Tri-F	26.9	66.4
14a	2,4-Di-F	>100	25.3
14b	2,3,4-Tri-F	>100	>100
15a	2,4-Di-F	>100	>100
15b	2,3,4-Tri-F	>100	>100

[a] IC_{50}s were measured using SRB assay.
[b] IC_{50}s were measured using MTT assay.

Table 3
The antiproliferative activity of compounds **8f** and **11c** against various human cancer cell lines

Compounds	IC_{50} (μM)				
	A549[a]	HL60[b]	EC9706[b]	SMMC7721[b]	MCF-7[b]
8f	1.0	0.0022	8.4	1.9	24.2
11c	0.4	2.9	0.44	4.6	14.2

[a] IC_{50}s were measured using SRB assay.
[b] IC_{50}s were measured using MTT assay.

of hydrogen (**9a**) or fluorine (**9c**), completely eliminated antiproliferative activity (IC_{50} >100 μM, A549; IC_{50} >100 μM, HL60), which indicated that this position would not tolerate large substituents. Next, the entire aniline moiety was replaced with alkylamino groups (**10**). In this series, only the ethylamino analog **10b** (IC_{50} = 0.3 μM) showed 11-fold improvement in activity against the leukemia cancer cell line HL60 (vs **II**, IC_{50} = 3.3 μM). Furthermore, 5-methylation of the pyrimidine ring (**11**) significantly improved antiproliferative activity (vs **9** series). In particular, the IC_{50} of compound **11d** (3.4 nM) was a 1000- to 1400-fold improvement compared to the parent analogs (**II**, IC_{50} = 3.3 μM and **9d**, IC_{50} = 4.8 μM) and was 24-fold better than doxorubicin (IC_{50} = 0.082 μM) against the leukemia cancer cell line HL60. Even for the inactive compound **9b**, 5-methylation (**11c**) also greatly improved the antiproliferative activity against both cell lines (A549, IC_{50} = 0.4 μM; HL60, IC_{50} = 2.9 μM). Thus, in the **II** series, a 5-methyl substitution of the pyrimidine ring was optimal.

Generally, most of the compounds with a β-methoxyacrylate (Q^1) group (**9–11**) showed moderate to potent antiproliferative activity. However, further replacement of this toxophoric moiety with Q^2–Q^5 groups (**12–15**) greatly reduced compound potency against both cancer cell lines. This result demonstrated that the β-methoxyacrylate (Q^1) group is both necessary and an optimal substructure for maintaining compounds' activity.

To further investigate the antiproliferative activity, compounds **8f** and **11c** were selected as representatives of two structural classes (**I** and **II**) and tested in more human cancer cells commonly used: esophagus cancer cell line EC9706, liver cancer cell line SMMC7721 and breast cancer cell line MCF-7. As shown in Table 3, analog **8f** with high potency against leukemia cancer cell line HL60 displayed good selectivity over the other four cell lines (A549, EC9706, SMMC7721 and MCF-7). Analog **11c** exhibited both good activity and selectivity against lung (A549) and esophagus (EC9706) cancer cell lines over cell lines HL60, SMMC7721 and MCF-7.

In this Letter, in order to develop effective chemotherapeutic anticancer drug candidates, we designed and synthesized a series of new strobilurin–pyrimidine derivatives (**8–15**) based on our previously discovered antiproliferative compounds **I** and **II**. Their biological profiles were evaluated with two human tumor cell lines (A549 and HL60), using the SRB and MTT assays, respectively, and most of the analogs possessed moderate to potent antiproliferative activity. The SAR investigation indicated that compounds containing a 5-cyclic fused pyrimidine, fluorinated aniline, and/or 5-methyl-6-trifluoromethyl-pyrimidine substructures possessed good activity. Meanwhile, two potent candidates (**8f**, IC_{50} = 2.2 nM and **11d**, IC_{50} = 3.4 nM) were identified with nanomolar antiproliferative activity against leukemia cancer cell line HL60, which represents a 1000- to 2500-fold improvement in activity compared to the parent compounds **I** (IC_{50} = 5.5 μM) and **II** (IC_{50} = 3.3 μM), and 20- to 30-fold greater potency than the chemotherapy drug doxorubicin (IC_{50} = 0.082 μM). Further evaluation indicated that analogs **8f** and **11c** possess good selectivity against HL60 and A549/EC9706 cell lines, respectively. This study provides strong impetus to further develop these strobilurin–pyrimidine analogs as potential antitumor agents for the treatment of leukemia.

Acknowledgments

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Supplementary data

CCDC 893933 contains the supplementary crystallographic data of compound **9b**. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.045.

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