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Discovery of (7-aryl-1,5-naphthyridin-2-yl)ureas as dual inhibitors of ERK2 and Aurora B kinases with antiproliferative activity against cancer cells

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ABSTRACT

A novel series of (7-aryl-1,5-naphthyridin-2-yl)ureas was discovered as dual ERK2 and Aurora B kinases inhibitors. Several analogues were active at micromolar and submicromolar range against ERK2 and Aurora B, associated with very promising antiproliferative activity toward various cancer cell lines. Synthesis, structure activity relationship and docking study are reported. In vitro ADME properties and safety data are also discussed.

Signal transduction pathways have long been recognized for their central role in the regulation of mammalian cellular programs in response to extracellular cues.[1,2] A key player in this kinase-signaling cascade from growth factors to the cell nucleus is the mitogen-activated protein kinase (MAPK) pathway.[3] The MAPK pathway comprises several key signaling components and phosphorylation events that play an important role in tumorigenesis. These activated kinases transmit extracellular signals that regulate cell growth, differentiation, proliferation, apoptosis and migration functions.[4] The MAPK pathway encompasses different signaling cascades of which the Ras–Raf–MEK-extracellular signal-regulated kinase 1 and 2 (ERK1/2) is one of the most dysregulated in human cancer.[5,6] Upon activation by growth factors, serum, cytokines and osmotic stresses, ERK can phosphorylate and regulate multiple substrates such as cytoskeletal proteins, kinases and transcription factors within various cellular compartments.

The pivotal role of the Ras/Raf/MEK/ERK MAPK pathway in multiple cellular functions underlies the importance of the cascade in oncogenesis.[7]

In light of the recent success in the clinical development of small molecule inhibitors of protein kinases, components of Ras/Raf/MEK/ERK MAPK cascade have been the subject of intense research and drug discovery efforts.[8–11] Moreover, blocking ERK activity through inhibition of upstream activators would inhibit all ERK functions, some of which may be normal metabolic processes.[12] It may, thus, be more beneficial to target the ERK protein directly, not only because this may accomplish more effective blockade of the ERK signaling pathway, but also because this would raise the possibility of blocking ERK interactions with specific substrates, so that only abnormal cell functions are inhibited.

Aurora serine/threonine kinases (Aurora-A, Aurora-B, and Aurora-C) are vital component playing a critical role in regulating many of the processes that are pivotal to mitosis, and they are required for healthy cell growth and proliferation.[13] These proteins are overexpressed in multiple human tumor types, there has been considerable interest in developing Aurora kinase inhibitors as antitumor agents. A number of small-molecule Aurora kinase inhibitors have been reported and there are several compounds currently in Phase I/II clinical trials for cancer.[14]

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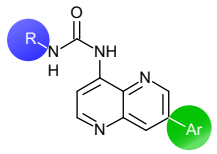
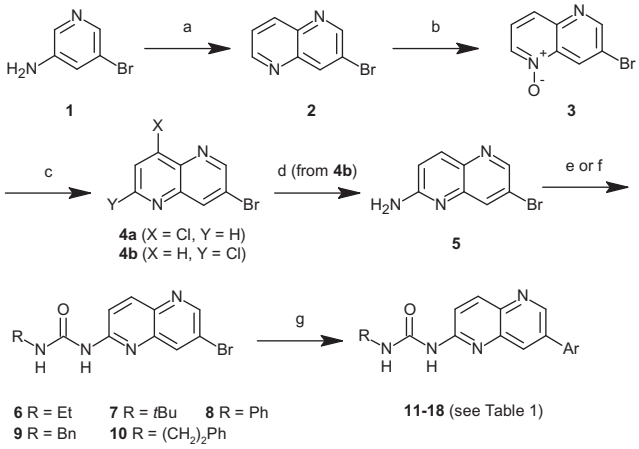


Figure 1. Structure of (7-aryl-1,5-naphthyridin-4-yl)ureas, Aurora kinase inhibitors.



Scheme 1. Reagents and conditions: (a) glycerol, $FeSO_4{\cdot}7H_2O$, H_3BO_3, H_2SO_4, *m*-NO_2PhSO_3Na, 135 °C, 18 h, 50%; (b) *m*CPBA, CH_2Cl_2, rt, 18 h, 64%; (c) $POCl_3$, CH_2Cl_2, reflux, 2.5 h, 54% (**4a**) and 18% (**4b**); (d) NH_4OH, dioxane, sealed tube, 140 °C, 24 h, 85%; (e) RNCO, pyridine, sealed tube, 140 °C, 24 h, 70-92%; (f) (i) triphosgene, Et_3N, CH_2Cl_2, reflux, 1 h (ii) $Ph(CH_2)_2NH_2$, reflux, 2 h, 24% (for **10**); (g) arylboronic acid or ester, $Pd(PPh_3)_4$, Na_2CO_3, DMF/H_2O (10:1), 80 °C, 5–16 h, 35–78%.

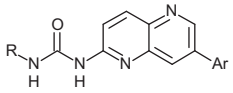
In our continued search for biologically active compounds that target kinases and cancer,[15] we previously reported that (7-aryl-1,5-naphthyridin-4-yl)ureas showed both Aurora A/B inhibitory activity in nanomolar range, and also displayed selectivity in an enzyme-based inhibition assay with a panel of seven kinases (Fig. 1).[16] Unfortunately, all the compounds suffered from lack of antiproliferative activity when tested toward five tumor cell lines.

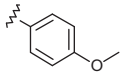
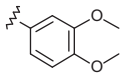
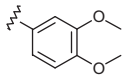
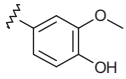
To develop this series of molecules more in depth, we considered modifying the position of the urea appendage on 1,5-naphthyridine ring from 4 to 2. This modification maintained inhibition of the Aurora B kinase, but also led to the inhibition of the Raf/MEK/ERK signaling pathway. In this paper, we describe the preliminary SAR and characterization of the resulting dual inhibitors.

As previously described, the synthesis of 7-bromo-2-chloro-1,5-naphthyridine **4b** started from commercially available 3-amino-5-bromopyridine **1**, which was converted into 3-bromo-1,5-naphthyridine **2** via the Skraup procedure (Scheme 1).[16,17]

Subsequent N-oxidation with 3-chloroperbenzoic acid and Meisenheimer chlorination using phosphorus(III) oxychloride furnished two isomers **4a** and **4b**. Compound **4a** was already published by our group as starting material for the design of 4,7-disubstituted 1,5-naphthyridines of biological interest.[16] Its regioisomer **4b** provided the suitable precursor for further pharmacomodulation at the positions 2 and 7 of the 1,5-naphthyridine ring and involving the same experimental conditions. In this purpose, selective amination of compound **4b**, by substitution of chlorine atom, was performed in the presence of ammonium hydroxide in sealed tube at 140 °C to give key heterocyclic amine **5** (Scheme 1). (7-Bromo-1,5-naphthyridin-2-yl)ureas **6**–**10** were prepared from azaheterocyclic amine **5** using various isocyanates, in pyridine at reflux or in two steps in the presence of triphosgene, triethylamine

Table 1
(7-Aryl-1,5-naphthyridin-2-yl)urea kinase inhibitors **11**-**18** used in this study and their selectivity profile



Compounds	R	Ar	IC_{50}[a] (μM)							
			ERK2	Aurora B	HIPK1	Pim1	KDR	TrkA	c-Abl	Yes
11	Et		1.13	2.23	>100	37.1	>100	>100	20.84	>100
12	Et		0.742 (>100)[b]	0.624 (0.136)[b]	13.6	>100	>100	>100	>100	41.8
13	*t*Bu		1.72 (>100)[b]	0.805 (0.714)[b]	>100	>100	>100	>100	>100	85.54
14	*t*Bu		0.702 (>100)[b]	0.227 (1.58)[b]	>100	>100	>100	80.63	>100	42.90
15	*t*Bu		0.429 31.60[b]	0.260 (0.112)[b]	>100	>100	>100	4.50	6.05	7.95
16	Ph		0.191 (>100)[b]	1.14 (1.26)[b]	>100	>31.6	>100	>100	>100	>100
17	Bn		0.030 (>100)[b]	0.579 (0.066)[b]	>100	>31.6	>100	>100	>100	>100
18	CH_2CH_2Ph		0.733 (>100)[b]	0.171 (0.086)[b]	>100	32.54	>100	12.67	>100	26.94

[a] Values are the mean of at least two independent determinations and are within ±15% SD.
[b] IC_{50} value of the corresponding 4,7-disubstituted isomer.[16]

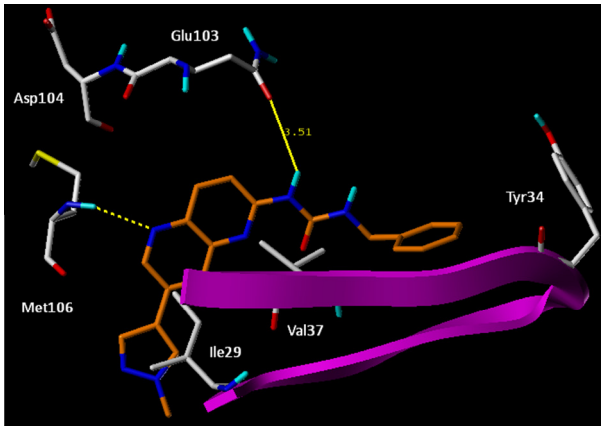
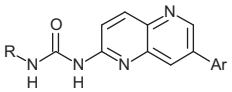


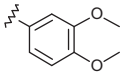
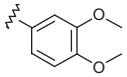
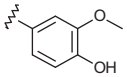
Figure 2. Docking solution of compound 17 into the ATP-binding site of human ERK2 (3I5Z.pdb).[25] Hydrogen bonds are indicated as yellow dotted lines. P-loop motif is presented as magenta ribbon.

and then adding the desired amine. Finally, aromatic moieties were introduced at the position 7 of the ring under a palladium-catalyzed Suzuki–Miyaura type reaction, using boronic acids or esters and leading to target compounds **11**–**18** in low to good yields.

(7-Aryl-1,5-naphthyridin-2-yl)ureas **11**–**18** were first tested for their inhibition toward a panel of eight cancer-related protein kinases (Table 1), which include mitogen-activated protein kinase (ERK2), serine/threonine kinases (Aurora B, HIPK1, Pim1),[18,19] receptor tyrosine kinases (KDR, TrkA)[20,21] and non-receptor tyrosine kinases (c-Abl, Yes).[22,23] Globally, the biological results showed that all the compounds **11**–**18** displayed good activities against Aurora B and ERK2 enzymes (IC_{50} values in the sub-micromolar to micromolar range) while no inhibition was found toward the other tested kinases. In particular, the pyrazolyl derivative **17** bearing a benzylurea at the position 2 of 1,5-naphthyridine ring exhibited the best inhibitory activity against ERK2 (IC_{50} value of

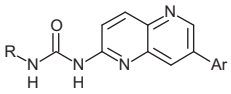
Table 3
Lipophilicity profile of (7-aryl-1,5-naphthyridin-2-yl)ureas **12**-**18**

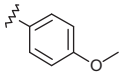
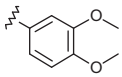
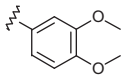
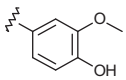


Compounds	R	Ar	CHI[a]	Log*D* pH 7.4 calcd[b]
12	Et		73.3	2.4
13	*t*Bu		88.8	3.2
14	*t*Bu		79.3	2.7
15	*t*Bu		76.1	2.5
16	Ph		73.3	2.4
17	Bn		69.4	2.2
18	CH_2CH_2Ph		71.6	2.3

[a] Determined by RP-UPLC.
[b] Calculated from CHI.

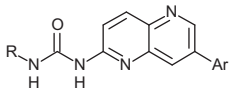
Table 2
(7-Aryl-1,5-naphthyridin-2-yl)urea kinase inhibitors **11**-**18** used in this study and their antiproliferative activity.

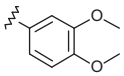
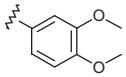


Compounds	R	Ar	Enzyme assays IC_{50}[a] (μM)		Cell proliferation assays—IC_{50}[a] (μM)				
			ERK2	Aurora B	HCT116	MDA-MB468	PC3	A549	U87MG
11	Et		1.13	2.23	15.88	>50	>50	>50	>50
12	Et		0.742	0.624	4.36	21.87	>50	>50	>50
13	*t*Bu		1.72	0.805	>50	>50	>50	>50	>50
14	*t*Bu		0.702	0.227	4.59	3.89	4.91	14.15	9.68
15	*t*Bu		0.429	0.260	0.651	1.96	0.671	1.00	>50
16	Ph		0.191	1.14	1.38	1.31	1.37	3.08	>50
17	Bn		0.030	0.579	0.191	>50	>50	>50	>50
18	CH_2CH_2Ph		0.733	0.171	1.20	1.80.	n.d.	n.d.	>50

[a] Values are the mean of at least two independent determinations and are within ±15% SD. n.d.: not determined.

Table 4
In vitro ADME properties and safety data of compounds **12**, **13** and **18**



Compounds	R	Ar	Solubility[a] (μM)	Mouse liver microsomes (% rem. after 1 h)	Mouse plasma (% rem. after 6 h)	Caco-2 permeability ab/ba[b] (10^{-6} cm s^{-1})	CYP3A4 inhibition (μM)	hERG binding (μM)
12	Et		5.7	45.4	n.d.	n.d.	n.d.	n.d.
13	*t*Bu		8.5	70.4	n.d.	n.d.	n.d.	n.d.
18	CH_2CH_2Ph		3.9	81.1	96.7	64/29	>100	>60

[a] Measured at pH 7.4, PBS medium + 1% BSA.
[b] In the presence of 1% BSA. n.d.: not determined. rem.: remaining.

30 nM), with an approximate 10-fold increase in potency compared to other compounds. In comparison to the previously described 4,7-disubstituted analogues which were inactive against ERK2,[16] their potency is maintained against Aurora B. This last observation is unclear since Aurora kinases contain a DFG motif able to adopt complex and unusual conformations and its possible involvement is still studying.[24]

The binding pose found by the docking program GOLD (GOLD version 4.0; CCDC, Cambridge, UK) for the most active compound **17** into the ATP pocket of human ERK2 is shown in Figure 2. A key hydrogen bond with the ATP hinge residue Met106 is observed while the urea moiety is directed toward the gatekeeper residue Gln103. Even if no additional hydrogen bond was done (d = 3.51 Å between NH and carbonyl groups), we can suppose that side-chain flexibility could be engaged to favour this specific type of interaction. Thus, as the gatekeeper residue is poorly conserved among other tested kinases, and is known to play a well-established role in determining the selectivity of kinase inhibitors, it is likely that the lack of inhibitory activities may be explained in part either by the hydrophobic nature of the corresponding residues from HIPK1 (Phe), Pim1 (Leu), KDR (Val) and TrkA (Phe) or shorter polar residues with c-Abl and Yes (Thr) that could not interact with the urea moiety. Finally, the benzyl group should also probably contribute to the affinity by making closer hydrophobic interactions with Tyr34 and Val37 residues in the P-loop motif.

Compounds **11**–**18** were tested for their antiproliferative activity against five cancer cell lines (HCT116 colon, MDA-MB468 breast, PC3 prostate, A549 NSCLC and U87MG CNS: Table 2). In a general trend, [7-(1-methyl-1*H*-pyrazol-4-yl)-1,5-naphthyridin-2-yl]ureas **15**–**18** proved to be very active, except on U87MG cell line, displaying micromolar IC_{50} values. Interestingly, benzylurea **17** was selective of HCT116 cell line with a very promising IC_{50} value of 0.191 μM and this compound was also the most active against ERK2 kinase (IC_{50} = 30 nM). (1,5-Naphthyridin-2-yl)urea counterparts **11**–**14**, without pyrazolyl moiety, remained inactive or showed slight antiproliferative potency toward the tested tumor cell lines.

In order to determine lipophilicity profile of the naphthyridine ureas **12**–**18**, their partition coefficient at pH 7.4, expressed as Log*D*, was directly derived from measurements of their chromatographic hydrophobicity index (CHI) (Table 3).[16,26] Log*D* ranged from 2.2 to 3.2 and were found acceptable for cell permeability.

Compounds **12**, **13** and **18** were profiled for PBS aqueous solubility and mouse liver microsomal stability (Table 4).[16] Their aqueous solubility (3.9–8.5 μM) was very weak and constituted a deleterious parameter in terms of suitable PK profile. Stability tests disclosed that the three analogues showed moderate (45.4%, **12**) to good (70.4%, **13** and 81.1%, **18**) metabolic stability in liver microsomes. In addition, urea **18** exhibited an excellent metabolic stability of 96.7% in mouse plasma. Moreover, cell permeability in Caco-2 cells[27] (Table 4) indicated that compound **18** had a very good cell permeability associated with a reduced efflux (0.45) (ratio = 0.45, [b→a/a→b]). Safety testing showed no inhibition of cytochrome P450, CYP3A4[28] and no undesired activity on hERG[29] channel for compound **18**.

In summary, a new series of dual ERK2 and Aurora B kinases inhibitors has been developed based on (7-aryl-1,5-naphthyridin-2-yl)urea scaffold. The molecules displayed antiproliferative activity in various cancer cells in the low micromolar and submicromolar range, a result that brings a significant improvement over previously published 4,7-disubstituted isomers which remained inactive in this assay.[16] The notion of inhibiting multiple kinases involved in tumor progression with a single small molecule is well established.[30] In this context, additional ERK2 inhibition led us to design very promising antitumor agents and this SAR study provides a useful starting point for lead optimization. In particular, efforts will be made to enhance the solubility of these poorly water-soluble compounds. To validate the concept of synergistic effect of the observed dual inhibition, additional pharmacological experiments are in progress to prove if the observed antiproliferative effect can be assigned to key signaling events of the respective target inhibition.

Acknowledgment

We thank AtlanChim Pharma Company for lipophilicity determination.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.06.078.

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