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Synthesis and SAR of new pyrazolo[4,3-*h*]quinazoline-3-carboxamide derivatives as potent and selective MPS1 kinase inhibitors

Marina Caldarelli *, Mauro Angiolini, Teresa Disingrini, Daniele Donati, Marco Guanci, Stefano Nuvoloni, Helena Posteri, Francesca Quartieri, Marco Silvagni, Riccardo Colombo

Nerviano Medical Sciences srl, Business Unit Oncology, Viale Pasteur 10, 20014 Nerviano, MI, Italy

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ABSTRACT

The synthesis and SAR of a series of novel pyrazolo-quinazolines as potent and selective MPS1 inhibitors are reported. We describe the optimization of the initial hit, identified by screening the internal library collection, into an orally available, potent and selective MPS1 inhibitor.

MPS1 (Monopolar Spindle-1 kinase), also known as TTK, plays critical roles in the proper execution of mitosis, is frequently over-expressed in human tumors, and is required for tumor cell proliferation.[1–3] Specifically, MPS1 kinase activity regulates the spindle assembly checkpoint (SAC), which in mitosis is responsible for the correct chromosome segregation, and its inhibition leads to rapid cell division with massive aneuploidy not compatible with cell survival.[4–7] Selective inhibitors of the target may provide an innovative therapy for the treatment of tumors and SAC inhibition which could be a way to selectively target aneuploid tumor proliferation.[8,9] We report the synthetic process and the structure–activity relationship that led from the initial hit (**1**) to the identification of a potent, orally available and highly selective MPS1 inhibitor, which shows tumor growth inhibition in xenograft mouse models.[10]

The tricyclic core ring system of 4,5-dihydro-pyrazolo[4,3-*h*]-quinazoline (Fig. 1) emerged as one of the most promising scaffolds from our HTS campaign for the identification of MPS1 inhibitors. This core was previously identified as an inhibitor of other cell cycle kinases like Aur-A, CDK2[11] and PLK1.[12] Thus, our initial efforts were directed at gaining selectivity for MPS1 versus other kinases that had shown high affinity for this chemical class. According to its enzymatic activity against MPS1 (IC_{50}: 0.500 μM), compound **1** (Table 1) was considered a reasonable starting point to undertake a medicinal chemistry program. Compound **1** was previously prepared and reported as a potent inhibitor of other cell cycle kinases (Aur-A, CDK2/A and PLK1)[11,12] with moderate anti-proliferative activity against the ovarian cancer cell line A2780 (IC_{50}: 0.500 μM) indicating its cell permeability.

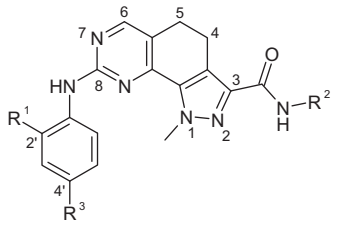

Figure 1. 4,5-Dihydro-pyrazolo[4,3-*h*]quinazoline-3-carboxamide core.

The envisioned optimization strategy was first to modify the different parts of the template independently and then to combine the best substituents into a single compound that would possess an improved overall profile. The template explorations were mainly focused on the *ortho*-residue (R^1), on the solubilizing portion (R^3) on the phenyl ring and on the amide (R^2) of the core (Fig. 1).

The synthesis of all the compounds was adapted from previously described methods[11,12] and is outlined in Schemes 1 and 2. Enaminone **26**, iodoquinazoline **27** and aminoquinazoline **28** were prepared according to already reported methods[11,12] starting from commercially available 1,2-cyclohexanedione. Compound **26** was condensed with phenylguanidine in DMF to form the desired

* Corresponding author. Tel.: +39 331 581551; fax: +39 331 581347.
E-mail address: marina.caldarelli@nervianoms.com (M. Caldarelli).

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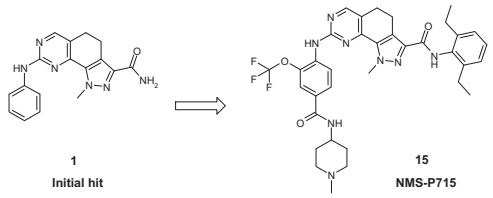
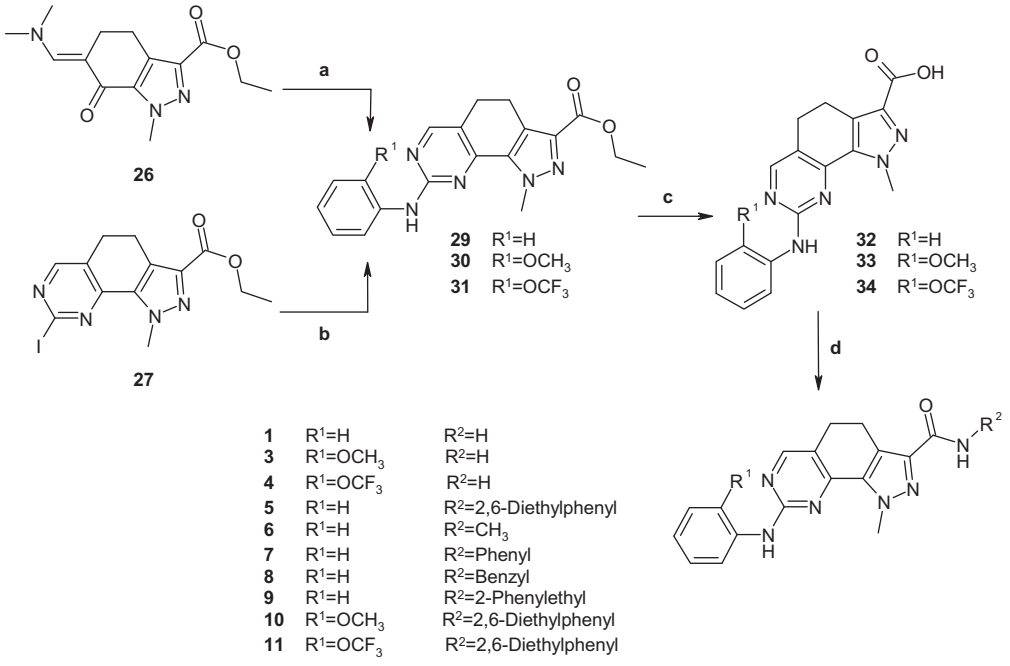
Table 1
Results for compounds **1**–**11**



Compd	R^1	R^2	IC_{50}[a] (μM) MPS1	IC_{50}[a] (μM) Aur-A	IC_{50}[a] (μM) CDK2/A	IC_{50}[a] (μM) PLK1
1	–H	–H	0.500	0.050	0.002	0.068
2	$-CH_3$	–H	0.241	0.605	0.003	0.015
3	$-OCH_3$	–H	0.066	0.607	0.090	0.042
4	$-OCF_3$	–H	1.218	>10	>10	0.117
5	–H	2,6-Diethylphenyl	0.530	0.152	>10	>10
6	–H	$-CH_3$	2.170	0.312	0.019	4.215
7	–H	Phenyl	>10	>10	0.574	>10
8	–H	Benzyl	>10	0.374	1.053	>10
9	–H	2-Phenylethyl	>10	0.677	0.697	>10
10	OCH_3	2,6-Diethylphenyl	0.525	3.168	>10	>10
11	OCF_3	2,6-Diethylphenyl	0.823	>10	>10	>10

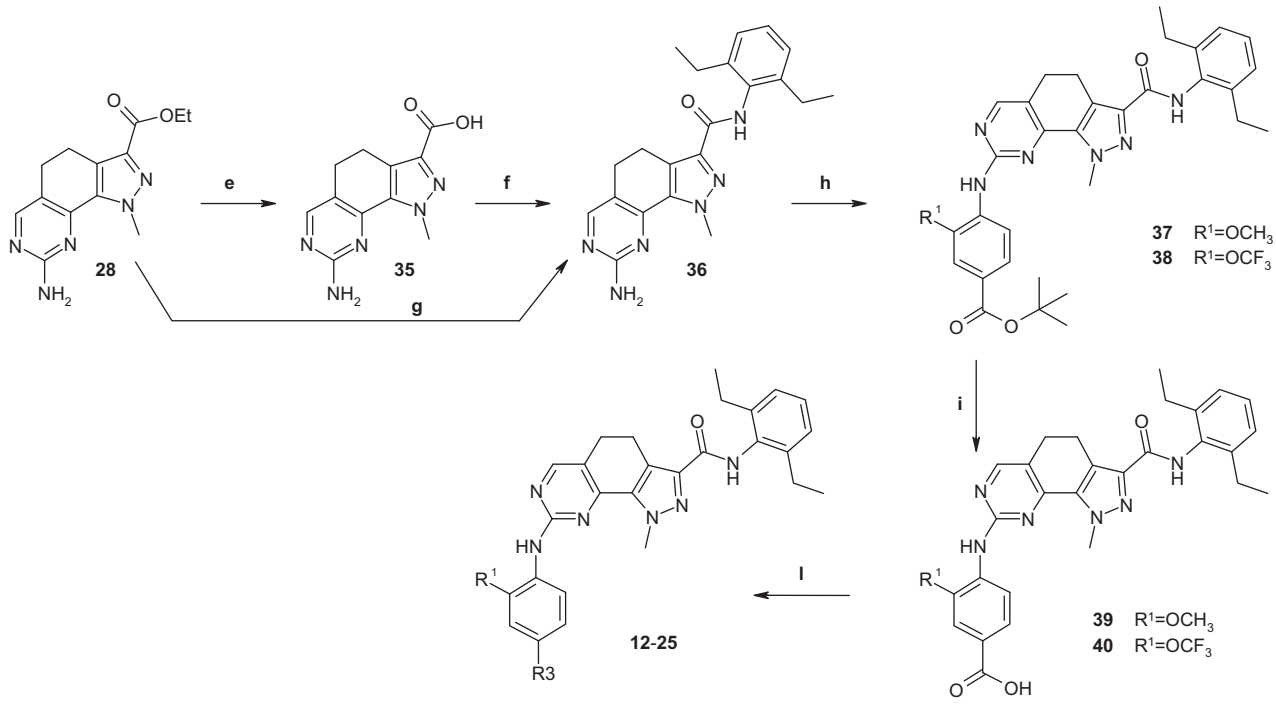
[a] Values are means of two or more experiments, standard deviation is <20%.



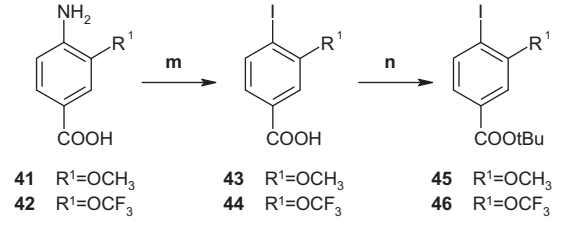
Scheme 1. Synthesis of final compounds **1** and **3**–**11**. Reagents and conditions: (a) phenylguanidine carbonate, DMF, 110 °C, 72%; (b) anilines, $Pd(OAc)_2$, (±)-BINAP, K_2CO_3, DMF 80 °C, 48–54%, (c) KOH/EtOH, reflux, quant.; (d) amines, EDC, HOBt, DMF, DIPEA, rt, 60–93%.

pyrimidine **29** (Scheme 1). Alternatively, different aromatic rings at position 8 of the scaffold were inserted by Buchwald–Hartwig coupling between iodo-intermediate **27** and substituted anilines using $Pd(OAc)_2$ and (±)-BINAP. Final amides **1** and **3**–**11** were obtained by hydrolysis of ethyl esters with KOH in EtOH to give acids **32**–**34**, followed by condensation with amines in the presence of HOBt and EDC in DMF. Compound **2** was prepared from iodoamide-quinazoline as previously described.[12] *Para*-substituted amides (R^3, Fig. 1) were prepared starting from intermediate **28** (Scheme 2) which was hydrolyzed to the corresponding acid **35** followed by amidation using 2,6-diethylaniline, HOBt and EDC in DMF to give **36**. Alternatively, compound **36** was obtained by ester aminolysis using 2,6-diethylaniline and $NaN(TMS)_2$ in THF.[13] 4-*t*-Butoxycarbonylaryl moieties were inserted by Buchwald–Hartwig coupling on quinazoline **36** with the suitable iodo-derivatives **45**–**46** in the presence of $Pd_2(dba)_3$ and X-Phos in dioxane. Finally, *t*-butyl esters **37**–**38** were hydrolyzed to acids **39**–**40**, which were subsequently converted, in the presence of amine, TBTU and DIPEA in DMF, to the corresponding final compounds **12**–**25** (Scheme 2). The iodo-intermediates **45**–**46** were in turn prepared from commercially available anilines **41**–**42** using KI and $NaNO_2$, and then protecting the carboxylic group of **43**–**44** under standard conditions (Scheme 3).

We observed the effect of the *ortho*-substitution (R^1, Fig. 1) on the phenyl ring (Table 1) and observed that, while the methyl-group (**2**) decreased the activity on Aur-A only, the methoxy-group (**3**) provided an increased inhibitory efficacy towards MPS1 if compared to CDK2/A and Aur-A but still maintained activity against



Scheme 2. Synthesis of final compounds **12**–**25**. Reagents and conditions: (e) KOH/EtOH, reflux, quant.; (f) 2,6-diethylaniline, EDC, HOBt, DMF, DIPEA, rt, 70%; (g) 2,6-diethylaniline, $NaN(TMS)_2$, THF, 0 °C, 80%; (h) 45–46%, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, dioxane, 90 °C, 85–88%; (i) TFA, DMF, rt, quant.; (l) amines, TBTU, DMF, DIPEA, rt, 72–88%.



Scheme 3. Synthesis of intermediates **45** and **46**. Reagents and conditions: (m) KI, $NaNO_2$, HCl, 0 °C, 46–75%; (n) Boc_2O, *t*-BuOH, DMAP, DCM, reflux, 62–91%.

PLK1. Replacement of the methoxy- with trifluoromethoxy-substituent (**4**) instead, abolished the activity on Aur-A and CDK2/A but also the inhibitory effect towards PLK1 and MPS1 was reduced (Table 1).

We then analyzed another portion of the molecule and observed how the modulation of the amide in position 3 of the core (R^2, Fig. 1) influenced both potency and selectivity. The introduction of 2,6-diethylphenylamide group on the scaffold resulted in a significant improvement in selectivity. Specifically compound **5**, was equipotent as **1** against MPS1, but was completely inactive on CDK2/A and PLK1 with a threefold lower activity against Aur-A than **1**. By contrast, replacement of the primary amide with other secondary alkyl amides did not show the same effect. For example, the introduction of a methyl (**6**) decreased the activity about 2–4-fold on all kinases except for CDK2/A. Other groups such as phenyl (**7**), benzyl (**8**) and phenylethyl (**9**) were instead not active on MPS1.

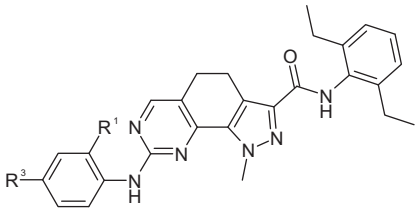
Having found favorable substitution elements for both the amide and the phenyl ring system, we next combined the best modifications and verified their compatibility. Compounds **10** and **11** (Table 1) were both active on the target and highly selective towards our target kinase MPS1. In spite of compound **10** being more potent than **11**, the trifluoromethoxy moiety gave a cleaner profile versus the kinases analyzed. In addition, compound **10** and **11** showed moderate cellular activity against A2780 (IC_{50}: 0.541 and 2.858 μM, respectively), but in both cases displayed low solubility of at <1 μM.

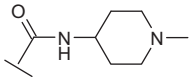
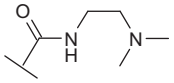
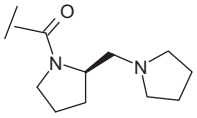
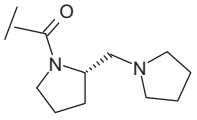
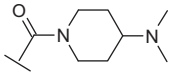
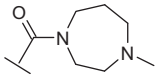
In order to improve solubility, we then started an exploration of solubilizing groups both on the *o*-methoxy and on the *o*-trifluoromethoxy series. Although the crystal structure of an inhibitor bound to MPS1 was not available during these investigations, we assumed (as was later confirmed),[10] a similar binding mode and orientation in the ATP pocket to the one observed for other kinases.[12,13] For this reason, we explored the solvent accessible region introducing several solubilizing groups at the 4′-position of the phenyl ring in position 8 of the tricyclic scaffold.

The introduction of primary amides at position 4′ (R^3, Fig. 1, **12**–**13**) increased the activity on the target while maintaining selectivity versus other kinases. All the other compounds described in Table 2 (both secondary and tertiary amides) were active in the range of 20–400 nM. A completely selective profile was observed for compounds bearing the trifluoromethoxy-group (**13**, **15**, **17**, **19**, **21**, **23** and **25**), as opposed to the corresponding methoxy-analogs (**12**, **14**, **16**, **18**, **20**, **22** and **24**). The presence of an asymmetric center on the amide portion had no impact on activity against MPS1 (**18** vs **20** and **19** vs **21**). The influence on solubility at pH 7 was not predictable, but methoxy-derivatives were, in general, more soluble than the corresponding trifluoromethoxy compounds, except for **14** and **15**. Moreover, compound **15** proved to be highly selective against a panel of more than 60 kinases.[10]

Compounds that showed reasonable potency against MPS1, selectivity and cell growth inhibition against a human tumor cell line (A2780), were then selected for further evaluation and tested in mechanism of action studies (MoA). Nocodazole arrested U2OS osteosarcoma cells were then treated, confirming the capability of our MPS1 inhibitors to promote mitotic override[10] (Table 2). Among the best compounds which showed, together with solubility, complete selectivity, good cellular activity and MoA, **15** and **25** were selected and further analyzed in preliminary pharmacokinetic (PK) experiments (Table 3). While compound **25** showed a high iv clearance and a low oral exposure, compound **15**

Table 2
Results for compounds **12–25**



Compd	R^1	R^3	IC_{50}[a] (μM) MPS1	IC_{50}[a] (μM) Aur-A	IC_{50}[a] (μM) CDK2/A	IC_{50}[a] (μM) PLK1	IC_{50}[a] (μM) A2780	IC_{50}[a] (μM) MoA	Solubility (μM) pH7
12	OCH_3	$-CONH_2$	0.036	0.768	5.568	3.034	0.135	0.004	<1
13	OCF_3		0.140	>10	>10	>10	0.298	0.16	1
14	OCH_3		0.084	1.450	>10	0.237	0.150	0.016	3
15	OCF_3		0.182	>10	>10	>10	1.000	0.032	45
16	OCH_3		0.033	0.750	>10	1.652	0.101	0.012	44
17	OCF_3		0.130	1.376	>10	>10	0.771	0.11	27
18	OCH_3		0.103	1.376	>10	>10	0.083	0.011	110
19	OCF_3		0.391[b]	>10	>10	>10	0.422	0.21	65
20	OCH_3		0.072	0.491	>10	>10	0.073	0.013	96
21	OCF_3		0.202[b]	>10	>10	>10	0.407	0.26	69
22	OCH_3		0.019	0.484	>10	>10	0.083	0.002	116
23	OCF_3		0.206	>10	>10	>10	0.485	0.040	70
24	OCH_3		0.021	0.372	3.625	>10	<0.016	0.002	59
25	OCF_3		0.146	>10	>10	>10	0.244	0.032	46

[a] Values are means of two or more experiments, standard deviation is <20%.
[b] Single data.

Table 3
PK parameters for compounds **15** and **25**[a]

Compd	In vivo PK (mouse) 10 mg/Kg iv				In vivo PK (mouse) 10 mg/Kg os			
	$t_{1/2}$ (h)	CL (mL/min/kg)	AUC (μM h)	V_{ss} (L/Kg)	$t_{1/2}$ (h)	C_{max} (μM)	AUC (μM h)	F (%)
15	7.65 ± 0.83	10.0 ± 0.24	22.4 ± 0.81	5.45 ± 0.55	8.01 ± 1.32	0.600 ± 0.150	8.66 ± 2.09	37.9
25	1.61 ± 0.14	77.5 ± 17.8	2.9 ± 0.72	8.41 ± 1.42	2.92 ± 0.89	0.245 ± 0.12	0.64 ± 0.25	22.4

[a] $n = 3$ animals per study.

(NMS-P715) showed a good half life value and oral bioavailability. On the basis of its favorable PK profile, **15** was therefore selected for in vivo efficacy studies in mice bearing A2780 tumor xenograft. When dosed *per os*, daily at 90 mg/Kg for seven consecutive days, at the end of the treatment it showed 53% tumor growth inhibition with no sign of body weight loss.[10]

In summary, a series of 4,5-dihydro-[4,3-*h*]quinazoline-3-carboxamides was synthesized and evaluated as MPS1 inhibitors. Compound **11** was found to be a selective inhibitor with an IC_{50} of 820 nM. Introduction of a solubilizing group on the core scaffold through an amide linkage not only improved the solubility but also significantly increased the enzymatic inhibitory potency and the cellular activity against A2780 cell line. Among the compounds prepared, **15** (NMS-P715) was found to have favorable pharmacokinetic parameters, good potency and selectivity profile. On the basis of the above results, compound **15** was selected for efficacy studies and for the first time, the in vivo antitumor activity of an MPS1 inhibitor was demonstrated. These results support the use of selective MPS1 inhibitors for cancer therapy.

Acknowledgments

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Supplementary data

Supplementary data (experimental methods and characterization of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.122.

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