Bioorganic & Medicinal Chemistry Letters 19 (2009) 3586–3592

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery of the potent aurora inhibitor MK-0457 (VX-680)

David Bebbington, Hayley Binch, Jean-Damien Charrier, Simon Everitt, Damien Fraysse, Julian Golec, David Kay, Ronald Knegtel, Chau Mak, Francesca Mazzei, Andrew Miller, Michael Mortimore *, Michael O'Donnell, Sanjay Patel, Francoise Pierard, Joanne Pinder, John Pollard, Sharn Ramaya, Daniel Robinson, Alistair Rutherford, John Studley, James Westcott

Vertex Pharmaceuticals (Europe) Ltd, 88 Milton Park, Abingdon OX14 4RY, United Kingdom

ARTICLE INFO

Article history:
Received 24 March 2009
Revised 24 April 2009
Accepted 27 April 2009
Available online 3 May 2009

Keywords:
Aurora
Kinase inhibitor
SAR

ABSTRACT

The identification of a novel series of Aurora kinase inhibitors and exploitation of their SAR is described. Replacement of the initial quinazoline core with a pyrimidine scaffold and modification of substituents led to a series of very potent inhibitors of cellular proliferation. MK-0457 (VX-680) has been assessed in Phase II clinical trials in patients with treatment-refractory chronic myelogenous leukemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) containing the T315I mutation.

Cellular proliferation is a tightly regulated process. A number of protein kinases have been assigned as critical mediators of cell-cycle progression. The Aurora family of highly homologous serine/thronine protein kinases (consisting of Aurora-A, -B and -C) regulate many of the processes that are pivotal to the final stages of cell division or mitosis. Inappropriate completion of mitosis leads to genetic instability and often to cells that contain non-diploid DNA content, a common hallmark of cancer.[1] The Aurora kinases were first recognised in 1995[2] and since then there has been an increasing body of evidence linking Aurora-A and -B expression with cancer.[3] Though the Aurora family members are structurally similar their biological functions are distinct. These differences have been extensively reviewed.[4–8]

Aurora-A is involved in regulating entry into mitosis and in early mitotic events. Its roles involve the bringing together and assembly of important components for the process of cell division. This includes recruiting microtubule spindle components to the centrosome to enable centrosome maturation. After maturation the centrosomes migrate apart. This process and formation of the mitotic spindle is mediated by kinesin motor protein Eg5 an Aurora-A substrate. Aurora-A depletion often results in delayed entry into mitosis, defects in centrosome maturation and microtubule organisation resulting in disruption of spindle formation leading to mono- or multi-polar spindles. Aurora-B also plays multiple roles in cell-division. It is involved in chromosome condensation, spindle formation and subsequent attachment of microtubules to the middle of the chromosome (kinetochore). The fidelity of this process is controlled by the spindle checkpoint which in turn is regulated by Aurora-B. Only when the kinetochores are correctly attached can the final stage of cell division (cytokinesis) take place. Cells depleted of Aurora-B fail cytokinesis and prematurely exit mitosis without division, leaving polyploid cells. Aurora-C is less well studied. It has a localisation pattern during cell division similar to that of Aurora-B. Aurora-C is required for spermatogenesis, but conclusive proof that it controls cell cycle progression in tumour cells is lacking.

A number of studies have demonstrated that depletion or inhibition of Aurora-A or - B by siRNA, dominant negative kinase mutant or neutralising antibodies results in critical disruption of mitosis and a block in proliferation leading to cell death in human cancer cell-lines (recently reviewed in Ref. 8). These observations have highlighted the Aurora kinases as promising targets for anti-cancer therapy. In this Letter, we describe the discovery and optimisation of a new series of Aurora inhibitors that led to MK-0457 (VX-680), the first Aurora inhibitor to enter clinical trials. MK-0457 (VX-680) is an Aurora inhibitor that disrupts mitosis, inhibits proliferation and promotes apoptosis in cycling cells while leaving non-cycling cells unaffected.[9] Since the discovery of MK-0457 (VX-680), many other Aurora inhibitors have also progressed to clinical evaluation.[5,10–15]

The Aurora-A gene is located on chromosome 20q13.2 which is frequently amplified in multiple human cancers and Aurora-A over expression has been observed in many tumours.[16] With these considerations in mind we initiated an oncology project with Aurora-A as our target. A screening campaign against full-length Aurora-A

* Corresponding author. Tel.: +44 0 1235 438805.
E-mail address: mike_mortimore@vrtx.com (M. Mortimore).

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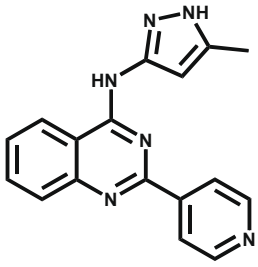
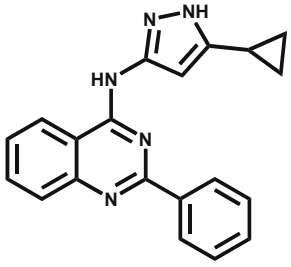
doi:10.1016/j.bmcl.2009.04.136

identified compound **1**, an amino pyrazole linked to a 2-substituted quinazoline, as a lead. Compound **1** is a potent inhibitor of Aurora-A (K_i = 81 nM) but also inhibits other kinases including Src (K_i = 181 nM) and GSK3β (K_i = 73 nM). These two kinases were used as counterscreens to gauge selectivity in the optimisation process; GSK3β was regarded as the kinase being most similar in sequence to the Auroras and Src kinase was used as a guide to general kinase inhibition. A co-complex crystal structure of compound **2**, a close analogue of compound **1**, with Aurora-A (Fig. 1) showed that the aminopyrazole provides three hydrogen bonds to the kinase hinge region and the 2-phenyl group on the quinazoline enters a lipophilic pocket, formed by the activation loop that is capped by Phe275 from the DFG motif and Trp277.[17] Since this lipophilic pocket did not appear to be a common feature of kinases, we hypothesised that maximising non-bonding interactions in this region would provide an opportunity for increasing potency and selectivity.



Compound **1**



Compound **2**

Examination of the crystal structure of the compound **2**/Aurora-A co-complex suggested that placing a linking atom between the quinazoline and the phenyl group of compound **2** would lead to more favourable interactions with the lipophilic pocket created by Phe275 and Trp277. In addition, this tactic would simplify the synthetic chemistry and allow greater scope for exploration. Of the four linkers examined, NH, NMe, O and S (Table 1), none gave a significant improvement in potency compared to compound **3** where a phenyl ring is directly attached to the quinazoline. However, all showed improvements in selectivity profile as judged by their cross-reactivity with Src and GSK3β. The nitrogen-linked compounds were found to inhibit a number of CyP_{450}s and were not considered further. The thioether **7** gave an encouraging selectivity

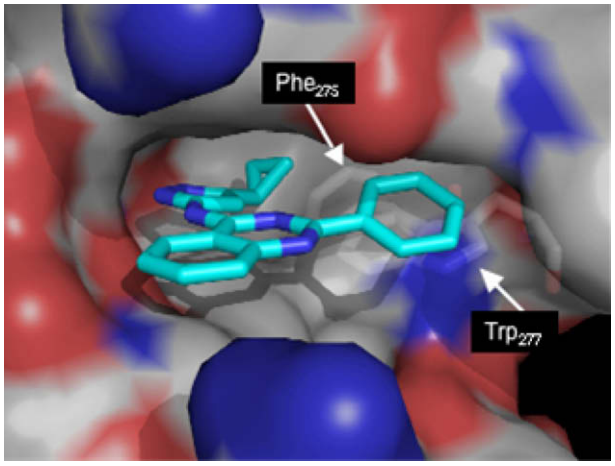
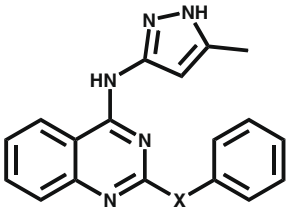


Figure 1. Co-complex crystal structure of Compound **2** in Aurora-A solved at 2.7 Å resolution with crystallographic R-factor = 0.26. The amino pyrazole makes three hydrogen bonds to the hinge region and the phenyl group enters lipophilic pocket towards the back of the ATP binding site made up of Phe 275 and Trp 277.

Table 1
Effect of linker between quinazoline and aromatic ring on kinase activity and selectivity



Compound	X	Aur-A K_i (nM)[18]	GSK3β K_i (nM)	Src K_i (nM)
3	Bond	58	22	81
4	NH	24	24	393
5	NMe	17	312	1059
6	O	36	51	1288
7	S	20	171	800

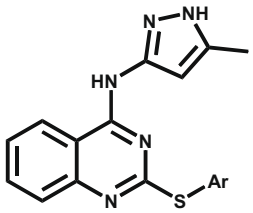
profile and this, coupled with the ease with which such compounds could be synthesised, prompted us to base our immediate future studies on the thioether system.

The introduction of simple lipophilic substituents onto the thiophenylethers was found to increase potency against Aurora-A by up to ten fold. In some cases, for instance the 3,4-dimethoxythiophenylether **17** and the 2-naphthylthioether **18** (Table 2), gains in selectivity against both Src and GSK3b were also observed. Perhaps more remarkably, some compounds showed good selectivity against Aurora-B as well. Despite many of these compounds giving K_i values of less than 10 nM against Aurora-A, none were able to inhibit the proliferation of Colo205 cells, as measured by ^{3}H thymidine uptake, at concentrations below 12 μM. This disappointing result was attributed to poor physical properties (e.g., the 2-naphthylthioether **18** has *c*Log *P* = 6.5) and low cellular penetration. Therefore, attempts were made to decrease the lipophilicity of the molecules while maintaining the overall shape of the 2-naphthylthioether **18**. Amides of 4-aminophenylthioethers were found to be suitable isosteres (compounds **21**, **23–26**, Table 3). In general, the amides retained or improved upon the potency against Aurora-A with respect to the naphthyl compound **18**, retained selectivity versus Src and GSK3β and inhibited proliferation of Colo205 cells at sub-μM concentrations.

Compound **21**, from the series of amides (Table 3), was found to have ADME properties ($T_{1/2} \sim$ 1.5 h, *F* = 40% in rats) considered sufficient to enable it to be used as a proof of principle compound in xenograft models of cancer and was therefore characterised more fully. Compound **21** was found to block proliferation of three different cancer cells lines with similar IC_{50} values of ~500 nM, inhibit Auroras-A, -B, and -C with K_i values of 4, 27 and 11 nM, respectively, and show >100-fold selectivity over 53 kinases in addition to Src and GSK3β. In addition, it was used to show that blocking of mitosis by an Aurora inhibitor results in cell death (IC_{50} for Colo205 cellular viability at 48 h using MTS staining = 8.5 μM). The cell death was due to apoptosis as shown by annexin V binding, DNA fragmentation ELISA and TUNEL staining. In a two-week xenograft experiment, the compound inhibited the growth of the MCF-7 tumour by 50% when administered at its maximum tolerated dose (MTD) (300 mg/kg bid po). Animal body weights were largely unaffected, though, as expected, total white blood cell counts were reduced. Taken together, these results increased our belief in Aurora kinase inhibition as a target for cancer therapy.

Although quinazoline compounds such as **21** had properties adequate for demonstrating pharmacological activity, better potency and improved physical properties, especially solubility, were required for clinical development. Examination of co-complex

Table 2
Effect of SAr substituents on kinase activity and selectivity



Compound	Ar	Aur-A K_i (nM)	Aur-B K_i (nM)	GSK3β K_i (nM)	Src K_i (nM)
7	Ph	20	590	171	800
8	2-ClPh	5	445	126	
9	3-ClPh	4	185	33	
10	4-ClPh	6	>1100	44	201
11	2,3-DiClPh	3	675	48	
12	2,4-DiClPh	2	>1100	8	
13	2,6-DiClPh	5	>1100	113	
14	3,4-DiClPh	2	>40	>15	>38
15	2-OMe	24	995	216	>1000
16	4-OMe	9	575	124	
17	3,4-DiOMePh	17	120	1168	2180
18	2-Naphthyl	1	>40	>150	>500

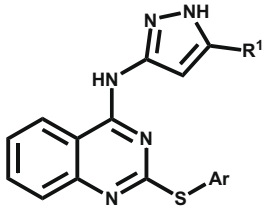
crystal structures led to the hypothesis that the lipophilic binding provided by the fused benzene portion of the quinazoline could be replaced by suitable substitution at the 6-position of a pyrimidine ring. The strategy of using a pyrimidine rather than a quinazoline as a core scaffold was particularly attractive in that it was expected to provide the scope for preparing more soluble molecules. Further lipophilic binding might be obtained by making better interactions in the pocket utilised by the 5-methyl group on the pyrazole of compound **21** or by increasing the size or nature of the amide group.

A series of compounds designed to optimise the substitution at the 5-position of the pyrazole (R^1 in Table 3) and the size of the amide on the thiophenyl ether group showed that while gains in potency might be achieved, a comparison between compounds **21**, **23–26** (Table 3) illustrates this point, it was usually at the expense of general kinase selectivity (data not shown). As a consequence, optimisation of pyrimidine-based Aurora inhibitors was focussed on 5-methylpyrazoles and amides of small aliphatic acids as the thiophenyl substituent.

Lipophilic substitution at the 6-position of the new pyrimidine scaffold, as illustrated by the sequence of inhibitors **27–35** in Table 4, led to compounds that were equal in potency against Aurora-A to quinazoline **21** but without gains in cellular potency. Introduction of heterocycles as the 6-substituent on the pyrimidine, as illustrated by the series of compounds **36–43**, had little effect in terms of potency against Aurora-A and only a marginal improvement against Aurora-B. However, a dramatic improvement in cellular potency was observed within the series. For instance, compound **37** with Aur-A K_i = 1.6 nM and Aur-B K_i = 8 nM inhibited Colo205 proliferation with IC_{50} = 400 nM, while compound **40**, with a similar Aurora inhibition profile inhibited cellular proliferation with IC_{50} = 24 nM. A 200-fold increase in cellular activity is observed for compound **43** over **37**, again with little difference between their respective ability to inhibit Aurora. While these results were broadly in line with the concept that that inhibition of Aurora-B rather than Aurora-A is more important for the inhibition of cell proliferation,[20,21] the remarkable increases in cellular potency were difficult to explain in terms of changes to physical properties or improvements in potency against Aurora-B.

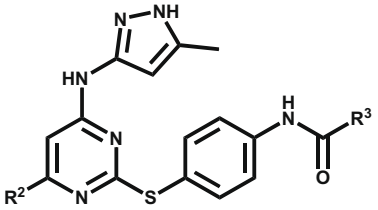
A co-complex crystal structure of compound **44** (VX-680/MK-0457) with Aurora-A showed the compound bound to a closed and, what might be considered to be, an inactive conformation of the enzyme.[22,23] The cyclopropyl group of the amide makes excellent interactions with a lipophilic pocket derived from Phe275 of the DFG loop (Fig. 2) that is not present in an open 'active' conformation. These observations led us to study the enzyme inhibition kinetics more deeply. It was noted that compounds that inhibited cellular proliferation most potently, the *N*-alkylpiperazine compounds, inhibited Aurora-B through a time dependent mechanism, with a long residence time for the compound on the enzyme (for compound **43**: Aurora-B $K_i^* = 0.8$ nM; $T_{1/2}$ = 8 h). Since the enzyme assay used for the structure activity studies assumed rapid equilibrium kinetics, the potency of such compounds on Aurora-B was underestimated. Extended analysis of the enzyme kinetics showed a two-step binding process. It is likely that the first step is the formation of a complex between the inhibitor and an open conformation of the active enzyme followed by a conformational change to deliver a tight binding complex. These inhibitors exhibit normal, rapid equilibrium, reversible kinetics with Aurora-A. The anomalies between enzyme K_i and Colo205 IC_{50} can now be explained, not in terms of Aurora-A affinity or lipophilicity, but cellular po-

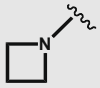
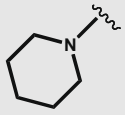
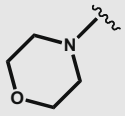
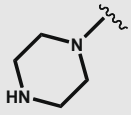
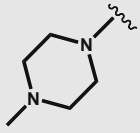
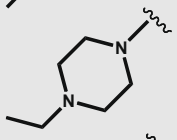
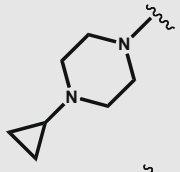
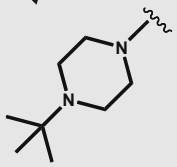
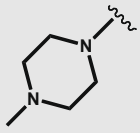
Table 3
Optimisation of S-naphthyl mimics for kinase activity and cellular potency



Compound	R^1	Ar	Aur-A K_i (nM)	Aur-B K_i (nM)	GSK3β K_i (nM)	Src K_i (nM)	Colo205 IC_{50} (μM)[19]
18	Me	2-Naphthyl	1	>40	>150	>500	>12
19	Me	4-(NHSO2Me)Ph	2	230	67	681	0.78
20	Me	4-(NHC(O)OtBu)Ph	9	250	>4000	>3000	2.87
21	Me	4-(NHC(O)Me)Ph	4	27	1034	1258	0.48
22	Me	4-(NMeC(O)Me)Ph	<1	105	17	796	5.77
23	Me	4-(NHC(O)Et)Ph	1	57	1500	720	0.83
24	Me	4-(NHC(O)cPr)Ph	<1	51	1314	461	0.91
25	H	4-(NHC(O)Me)Ph	24	>1100	605	277	2.20
26	cPr	4-(NHC(O)Me)Ph	<1	15	338	1735	0.21

Table 4
Effect of 6-pyrimidine substituents on kinase activity and cellular potency



Compound	R^2	R^3	*c*Log *P*	Aq. solubility at pH 7.4 (μg/ml)	Aur-A K_i (nM)	Aur-B K_i (nM)	Colo205 IC_{50} (μM)
27	H	Me	3.0	13.8	86	207	4.05
28	Me	Me	3.5	<1	18	220	2.00
29	Ph	Me	5.1		2.4	83	
30	Me	Et	4.0	4.2	5.1	153	0.72
31	CyPr	Et	4.4		3.9	59	1.42
32	tBu	Et	5.3	11.2	10	115	2.00
33	Ph	Et	5.6		<1	145	0.87
34	3-Py	Et	4.2	<1	3.9	52	0.47
35	4-Py	Et	4.2		<3	49	1.00
36		Et	4.0		2.4	23	0.73
37		Et	5.1	2.5	1.6	8	0.400
38		Et	3.7	5.4	1.7	14	0.135
39		Et	3.7	296	3.7	18	0.079
40		Et	4.1	63.4	1.6	20	0.024
41		Et	4.8	114.5	<1	25	0.024
42		Et	4.6	12.8	<1	9.5	0.075
43		Et	5.4	220.8	1.3	11	0.002
44		CyPr	4.3	6.8	0.6	18	0.019

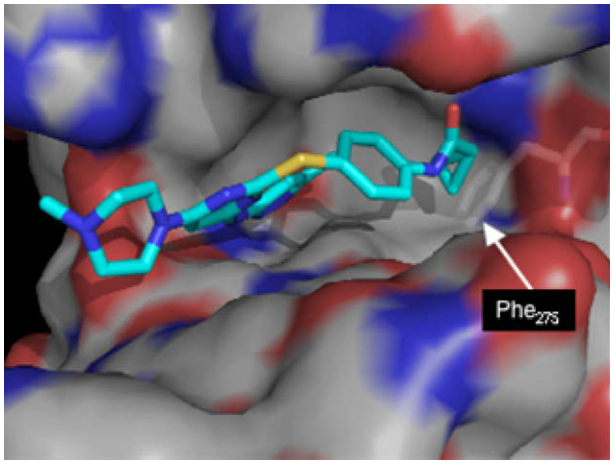
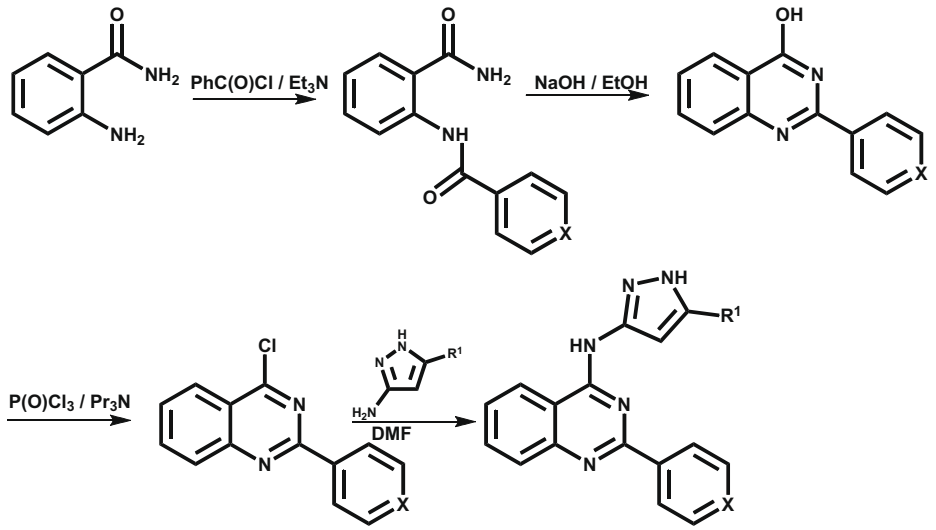


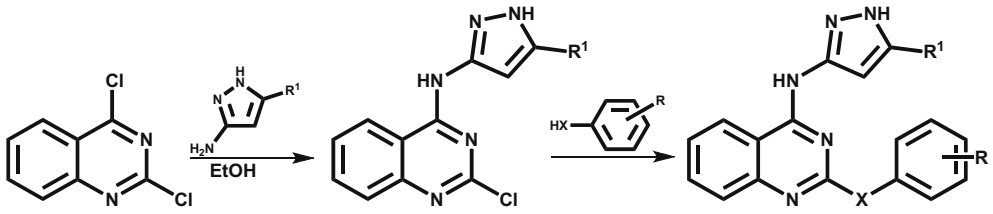
Figure 2. Co-complex crystal structure of compound **44**, MK-0457 (VX-680), in Aurora-A, solved at 2.9 Å resolution with crystallographic R-factor = 0.24, showing the cyclopropyl group of the amide packing against Phe 275.

tency correlated with the compounds' K_i^* and time-dependent kinetics with Aurora-B. A full description of the structural and kinetic studies together with the cellular activity and pharmacodynamic implications of such Aurora inhibitors will be reported elsewhere.

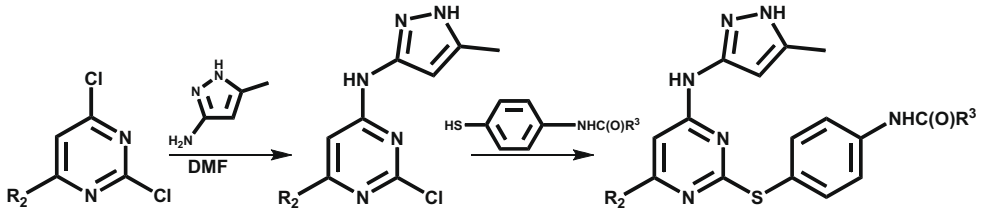
Compound **44** (MK-0457 (VX-680)) was considered to have the best combination of potency and pharmaceutical properties in this series of compounds and it was nominated for further development. It is a potent inhibitor of all three Aurora kinases with K_i of 0.6 nM against Aurora-A, 18 nM against Aurora-B and 5 nM against Aurora-C and K_i^* of 1.8 nM against Aurora-B. It shows selectivity over 190 other protein kinases although it does cross-react with a small number of unrelated kinases that are themselves interesting cancer targets, including Flt-3, Abl and the T315I Abl mutant, one of the most prevalent and resistant mutations of Abl.[22,24] The inhibition of these seemingly distinct kinases can be explained by compound **44** being able to bind to their closed, inactive conformations and so exploit lipophilic pockets that are not thought to be available in an open or active kinase conformation. MK-0457 (VX-680) is a potent anti-proliferative agent that is active



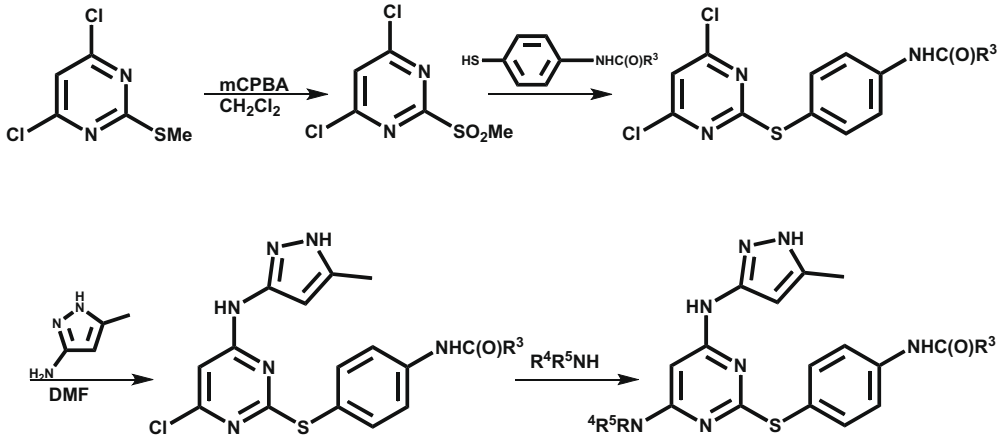
Scheme 1. X = N, CH. Preparation of quinazoline-based inhibitor compounds **1**–**3**.



Scheme 2. Preparation of quinazoline-based inhibitor compounds **4**–**26**.



Scheme 3. Preparation of 6-substituted pyrimidine-based inhibitor compounds **27**–**35**.



Scheme 4. Preparation of 6-substituted pyrimidine-based inhibitor compounds **36**–**44**.

against all cycling cells (IC_{50} value ranges from 15 nM to 113 nM). Treatment of proliferating cells with MK-0457 (VX-680) leads to accumulation of cells with 4 N DNA and in many cases extensive endoreduplication in the absence of cell division. A detailed description of its biological characteristics has been published.[9,25–29] MK-0457 (VX-680) has been assessed in a number of clinical studies. In particular it has been studied in a dose escalating Phase I/II study in refractory leukemias. It is interesting to note that of the 14 patients with chronic myelogenous leukaemia (CML) that were evaluated, 9 expressed the refractory T315I Abl mutation and significantly, of these, 8 showed a haematological or cytogenic response.[30–32] Although clinical studies on MK-0457 (VX-680) have now been stopped, alternative Aurora kinase inhibitors continue to be studied.

From an initial strategy of investigating the effect of inhibiting Aurora-A, the importance of inhibiting Aurora-B for preventing cellular proliferation and causing cell death became apparent. Through detailed inhibitor kinetic characterization and crystallographic investigation, the cellular potency and, to some extent, the selectivity profile of MK-0457 (VX-680) has been explained. It is fully expected that this understanding, together with what is learnt in the clinic, will be utilized in the design of further classes of Aurora inhibitors that will add to the armory of cancer therapies.

The synthesis of the Aurora inhibitors is shown in General Schemes 1–4 below. The quinazoline-based inhibitors described can be prepared according to Schemes 1 and 2.

The pyrimidine-based inhibitors described can be prepared according to Schemes 3 and 4.

Acknowledgement

The authors thank Dr. Graham Cheetham for determining the crystal structures.

References and notes

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19. Compounds were screened for their ability to inhibit cellular proliferation using Colo205 cells obtained from ECACC. Colo205 cells were seeded in 96 well plates and serially diluted compound was added to the wells in duplicate. Control groups included untreated cells, the compound diluent (0.1% DMSO alone) and culture medium without cells. The cells were then incubated for 72 or 96 h at 37 °C in an atmosphere of 5% CO_2/95% humidity. Three hours prior to the end of the experiment 0.5 μCi of 3H thymidine was added to each well. Cells were then harvested and the incorporated radioactivity counted on a Wallac microplate beta-counter. Dose response curves were calculated using either PRISM 3.0 (GRAPHPAD) or SoftMax Pro 4.3.1 LS (molecular devices) software.
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