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Pyrimido-oxazepine as a versatile template for the development of inhibitors of specific kinases

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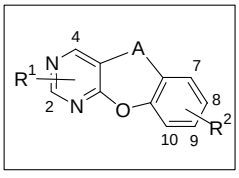
Abstract—Pyrimido-oxazepine based sub-micromolar inhibitors (**2**–**4**) for Aurora and FLT-3 were designed and synthesized. These preliminary results supported the potential use of pyrimido-oxazepines as a versatile template for developing specific kinase inhibitors.

In the past decade, the kinase targeted cancer therapy[1] has gained increasing momentum, leading to the launch of small molecule kinase inhibitors such as Gleevec,™[2] Irresa,™[3] and Tarceva.™[4] Both Iressa and Tarceva are quinazoline based chemical entities that target the epidermal growth factor receptor (EGFR). The quinazolines, like most of the known kinase inhibitors, are ATP competitive inhibitors. Due to the highly conserved ATP binding pockets, and their less conserved neighboring sites, it is feasible to modify the substituents on a common scaffold to target specific kinases. For example, quinazoline as a structural class has been reported as potent inhibitors of not only EGFR but also other kinases.[5] Recently, we disclosed the pyrimido-oxazepine **1** as potent EGFR inhibitors.[6] We therefore envisioned the possibility of extending this scaffold to inhibitors of other selected kinases. In this letter, we want to report our preliminary results that will serve to validate pyrimido-oxazepine, such as **1**–**4** (Fig. 1), as a new versatile scaffold for selected kinase inhibitors, upon modifications of its substitution pattern (e.g., R^1 and R^2).

Aurora[7] and FLT-3[8] were chosen as the biological targets based on an overall evaluation of their clinical relevance in cancer treatment and room for improvement among the known inhibitors currently in development.



1. A: -NH-CH_2-; R^1 = 4-NHAr; R^2 = H or alkoxy;
2. A: -NH-CH_2-, R^1 = 4-HN–(pyrimidine)–NHCOAr ; R^2 = H or alkoxy;
3. A: -NH-CH_2-; R^1 = Cl; R^2 = 8-NHCONHAr;
4. A: -N=CH-; R^1 = Cl; R^2 = 8-NHCONHAr

Figure 1.

Rational drug design and computer modeling generated oxazepines **2** as potential inhibitor for Aurora, as well as **3** and **4** for FLT-3.

A convergent synthetic route toward **2** was developed,[9] featuring a Buchwald-type coupling of weakly nucleophilic amines, such as **6**,[10] with 4-chloro oxazepine **5**[9] (Scheme 1). Coupling of independently prepared **5** and **6** expedited SAR studies aimed at examining the influence of substitutions on either aryl ring. The Aurora kinase inhibitory activity of **2** was determined with an Aurora A kit (Table 1).[11]

Results in Table 1 indicate that as expected, variations on R^2 had dramatic effects on the Aurora A inhibitory activity. When Ar was phenyl (**2a**–**e**), a methoxy

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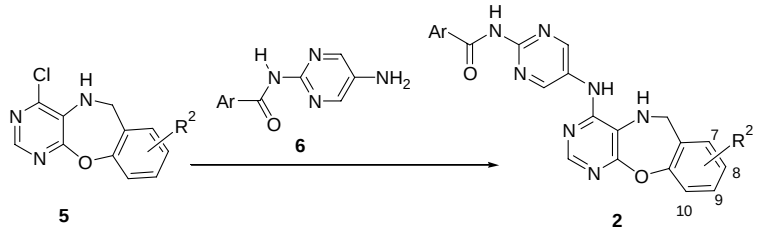
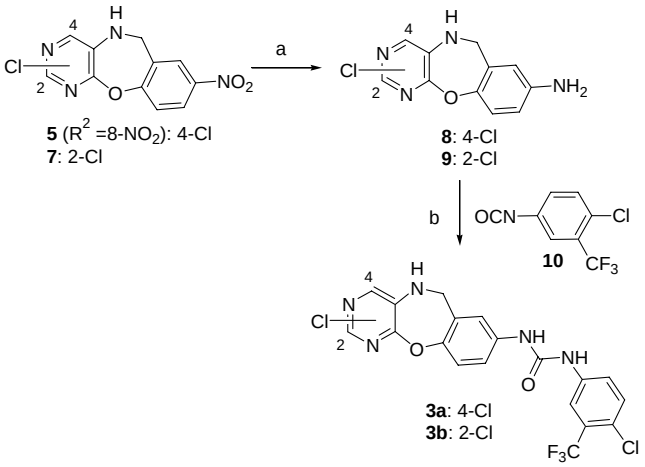


Scheme 1. Reagents and conditions: 0.25 equiv of $Pd_2(dba)_3$, 0.75 equiv of BINAP, *t*-BuONa, dioxane, 90 °C.

Table 1. In vitro inhibition of Aurora A by 2

Compound	R^2	Ar	Aurora A inhibition IC_{50}[a] (μM)
2a	H	Ph	1.9
2b	7-MeO	Ph	2.2
2c	8-MeO	Ph	0.97
2d	9-MeO	Ph	0.60
2e	10-MeO	Ph	>10
2f	8-MeO 9-MeO	Ph	0.47
2g	9-Et_2N	Ph	5.0
2h	H	*p*-MePh	3.7
2i	8-MeO	*p*-MePh	3.5
2j	8-MeO 9-MeO	*p*-MePh	1.2
2k	8-MeO	*m*-F-Ph	1.1
2l	9-MeO	*m*-F-Ph	0.32
2m	8-MeO 9-MeO	*m*-F-Ph	1.1
2n	9-MeO	*p*-F-Ph	0.33

[a] Average of three experiments with SD < 10%.



Scheme 2. Reagents and conditions: (a) In, NH_4Cl, EtOH, H_2O, reflux, 50–70%; (b) 1-chloro-4-isocyanato-2-trifluoromethyl-benzene (**10**), 80%.

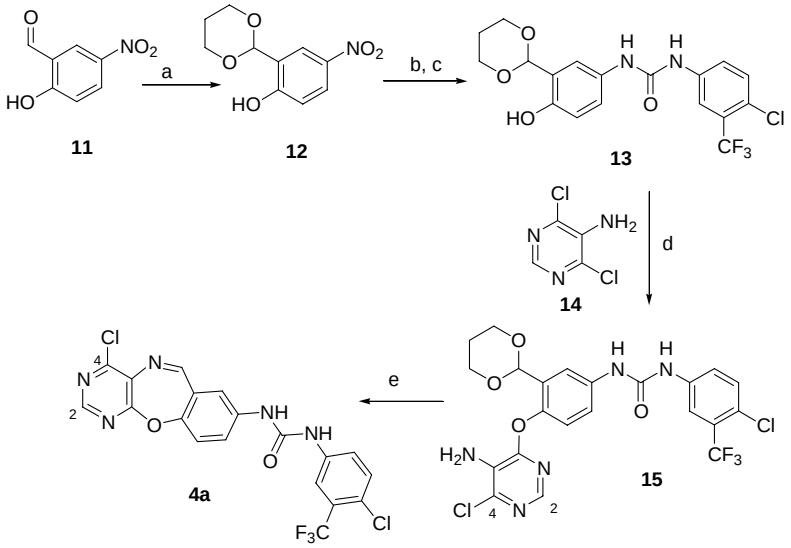
substituent at C-9 (**2d**) or C-8 (**2c**) was preferred. Compound **2f**, with 8,9-dimethoxy substitution, had an IC_{50} of 0.47 μM. Switching methoxy to a diethylamino substituent resulted in diminished activity (**2d** vs **2g**). When R^2 was 9-methoxy, a fluoro substituent at the *meta* position of the Ar group of the amide moiety improved the Aurora A inhibitory activity by about 1-fold (**2l** vs **2d**). A similar observation was made when Ar was substituted with a *para*-fluoro group (**2n** vs **2d**). In contrast, when R^2 was 8,9-dimethoxy, a *meta*-fluoro substituent resulted in a loss of activity (**2m** vs **2f**).

Encouraged by the results, and to further validate pyrimido-oxazepine as a versatile scaffold for kinase inhibition, we shifted our effort to the preparation of the oxazepine based FLT-3 inhibitors **3** and **4**. The preparation of **3** is illustrated in Scheme 2. The nitro groups in oxazepine **5** and **7**[9] were reduced with indium in the presence of ammonium chloride to afford anilines **8** and **9**, respectively, in moderate to good yields.[12] Since the early phases of SAR studies indicated that the preferred substituents on the terminal Ar moiety were *para*-chloro and *meta*-trifluoromethyl, **8** and **9** were then reacted with 1-chloro-4-isocyanato-2-trifluoromethyl-benzene (**10**) to afford **3a** and **3b** in good yields.

In order to obtain imine **4**, an alternative route as shown in Scheme 3 was developed. The aldehyde functionality of 2-hydroxy-5-nitro-benzaldehyde (**11**) was first protected as its 1,3-dioxane derivative to afford **12**.[13] The nitro group of **12** was then reduced under hydrogenation conditions, followed by condensation with the isocyanate **10** to give urea **13** in good overall yield. Addition of **13** to dichloro-pyrimidine **14** under basic conditions provided **15** in a 50% yield. The protected aldehyde moiety in **15** was then released in the presence of catalytic amount of *p*-toluene sulfonic acid (*p*-TsOH), followed by cyclization at 120 °C under vacuum, to afford imine **4a**. Although this route worked well for **4a**, which had a 4-Cl substituent, it failed to provide the corresponding 2-Cl isomer.

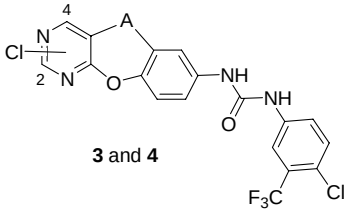
Oxazepines **3** and **4** were tested in a homogeneous time-resolved fluorescence (HTRF) assay,[14] and the results are summarized in Table 2. As expected, both **3b** and **4a** showed sub-micromolar activities against FLT-3, with **4a** having an IC_{50} of 0.45 μM. On the pyrimidine ring, the preferred substitution position was C-2 instead of C-4 (**3a** vs **3b**), indicating a possible future SAR direction. In a further phase of the SAR study, we also replaced the chlorides in **3a** and **4a** with various primary and secondary amines. However, this resulted in diminished FLT-3 inhibitory activity.[15]

Compound **4a** was also screened against other kinases including EGFR, KDR, ETK, FLT-1, IGF-1R, IR, c-Met, and FGF-1R. It showed modest activities against IR and KDR (IC_{50}s of around 9.5 μM), and no activities against the rest of the kinases even at 25 μM. Time course studies also excluded the possibility of **4a** acting



Scheme 3. Reagents and conditions: (a) $HO(CH_2)_3OH$, TsOH, 95%; (b) H_2/Pd(C), 80%; (c) 1-chloro-4-isocyanato-2-trifluoromethyl-benzene (**10**), 79%; (d) 4,6-dichloro-pyrimidin-5-ylamine (**14**), K_2CO_3, DMF, 50%. (e) 1—*p*-TsOH, H_2O, DME, 45 °C; 2—120 °C, vacuum, 60%.

Table 2. In vitro inhibition of FLT-3



Compound	Cl	A	FLT-3 inhibition IC_{50}[a] (μM)
3a	4-Cl	$-NHCH_2-$	13.7
3b	2-Cl	$-NHCH_2-$	0.9
4a	4-Cl	$-N{=}CH-$	0.45

[a] Average of three experiments with SD <10%.

as an irreversible FLT-3 inhibitor via covalent addition to its imine bond.

In summary, pyrimido-oxazepine based sub-micromolar inhibitors for Aurora and FLT-3 were designed and synthesized (**2**–**4**). These preliminary results supported the potential application of pyrimido-oxazepine as a versatile template for the development of inhibitors of specific kinases.

Acknowledgment

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in the kinase reaction was lowered from the suggested 50–2 μM.

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14. In vitro assay for FLT-3 kinase inhibition: FLT-3 tyrosine kinase inhibition is determined by measuring the phosphorylation of poly-Glu-Tyr-biotin (pGT-biotin, CIS Bio International) peptide in a Homogeneous Time-Resolved Fluorescence (HTRF) assay. Into a black 96-well Costar plate is added 2 μl/well of 25× compound in DMSO (final compound concentration in the 50-μl kinase reaction is typically 1 nM to 10 μM). Next, 38 μl of reaction buffer (25 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/ml BSA, and 2 mM DTT) containing 2 nmol pGT-biotin and 5 ng FLT-3 enzyme is added to each well. After 5–10 min pre-incubation, the kinase reaction is initiated by the addition of 10 μl of 10 μM ATP in reaction buffer, after which the plate is incubated at room temperature for 45 min. The reaction is stopped by the addition of 50 μl KF EDTA buffer (50 mM Hepes, pH 7.5, 0.5 M KF, 1 mg/ml BSA, and 100 mM EDTA) containing 0.23 μg/ml PY20K (Eu-cryptate labeled anti-phosphotyrosine antibody, CIS Bio International). After 30 min, 100 μl of 10 nM SV-XL (modified-APC-labeled Streptavidin, CIS Bio International) in KF buffer is added, and after an additional 2-h incubation at room temperature, the plate is read in a RUBYstar HTRF Reader.
15. Unpublished results.