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Imidazo[5,1-*f*][1,2,4]triazin-2-amines as novel inhibitors of polo-like kinase 1

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ABSTRACT

The synthesis and biological activities of imidazo[5,1-*f*][1,2,4]triazin-2-amines (imidazotriazines) as novel polo-like kinase 1 inhibitors are reported.

Polo-like kinases (PLK) are evolutionarily conserved serine/threonine kinases that regulate critical processes in the cell cycle.[1] The PLK family includes PLK1, PLK2 (SNK), PLK3 (PRK/FNK), and PLK4 (SAK). PLK1 plays key roles in the regulation of mitotic progression including mitotic entry, spindle formation, chromosome segregation and cytokinesis.[2] With the discovery of PLK1 as a critical regulator of mitotic progression, it has become a target of keen interest for the treatment of cancer.[3–5] Among all the PLK inhibitors under clinical investigation, dihydropteridinone BI 2536 and thiophene amide GSK461364 (Fig. 1) are the only two molecules that have demonstrated inhibition of PLK kinase activity.[5]

In order to discover inhibitors of PLK1, we conducted a focused screen of our kinase inhibitor collection and a high-throughput screen (HTS) of the GSK compound collection. From these screens, multiple chemical series were identified. Of particular interest were hits from a thiophene amide series and hits from an imidazotriazine series, exemplified by compound **1** and **2**, respectively (Fig. 2). Our efforts toward optimizing the thiophene amide series will be reported in due course.[6] Herein, we report the synthesis and biological activity of novel PLK1 inhibitors from the imidazotriazines series.[7]

In order to guide our SAR exploration, we built a PLK1 homology model based on the active confirmation of the protein PKA C-alpha. Docking of compound **2** into this model led to the proposed binding mode shown in Figure 3.[8] In this proposed model, the benzyl amine N–H and triazine 4-N were believed to form key hydrogen bond donating and accepting interactions with the hinge residue of Cys133 in PLK1, with both benzylic and phenyl groups facing towards the solvent region. Based on this model and success in other programs that target these regions of kinases,[9] we decided to ex-

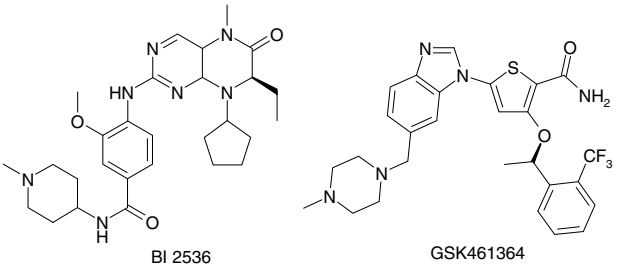

Figure 1. Structure of BI 2536 and GSK461364.

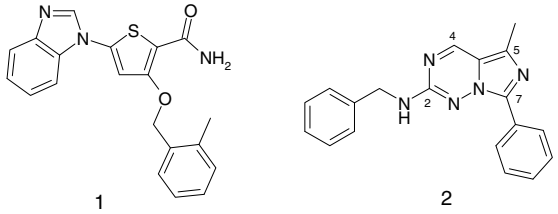

Figure 2. Structure of thiophene amide **1** and imidazotriazine **2**.

Abbreviations: PLK, polo-like kinase; SNK, serum-inducible kinase; PRK, proliferation-related kinase; FNK, fibroblast-growth-factor-inducible kinase; SAK, Snk akin kinase; HTS, high-throughput screening; SAR, Structure–activity relationships; CDK, cyclin-dependent kinase.

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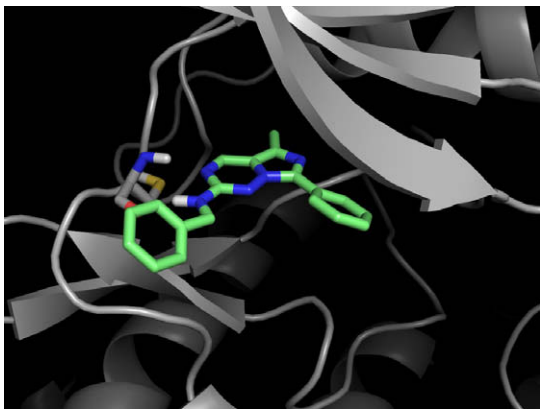
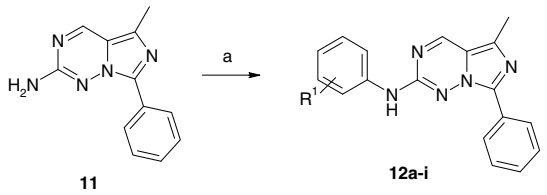


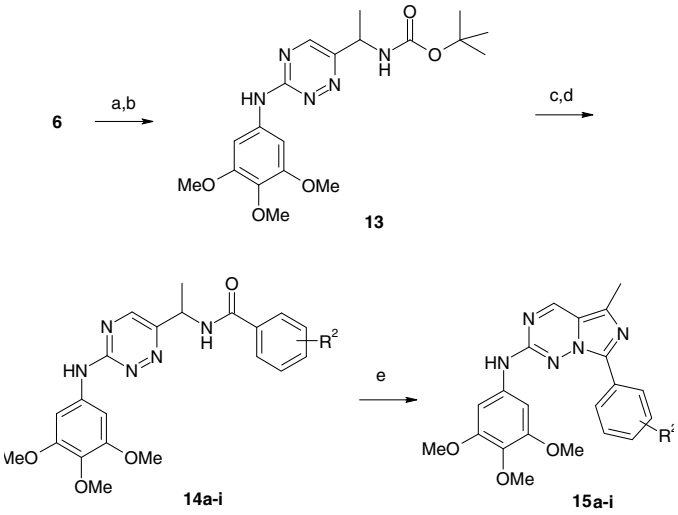
Figure 3. Imidazotriazine **2** in PLK1 homology model.

plore the structure–activity relationships (SARs) around the phenyl ring at position 7 and the benzyl group at position 2.

Synthetic access to the imidazotriazine ring system is rather limited.[10] In order to explore the SAR of this series we needed to develop a number of novel synthetic routes to allow better access to the targeted compounds. Herein, we described novel syntheses of imidazotriazines (Scheme 1–3). Alkyl amine substituted analogs **10a–c** were synthesized using reactions described in Scheme 1. Racemic *N*-Boc alanine **3** was coupled with *N*-methoxy-*N*-methylamine to form *N*-methoxy-*N*-methyl amide **4** which was then displaced by lithiated dithiane to form **5**. Condensation of **5** with thiosemicarbazide followed by cyclization in the presence of methyl iodide produced *tert*-butyl 1-[3-(methylthio)-1,2,4-triazin-6-yl]ethylcarbamate **(6)** and its regioisomer, *tert*-butyl 1-[3-(methylthio)-1,2,4-triazin-5-yl]ethylcarbamate **(7)** in a 6.7:1 mixture with desired **6** obtained in 17% isolated yield. Though low yielding, the condensation and cyclization step can be scaled up readily (600 g scale) to provide large quantity (74 g) of intermediate **6**. Deprotection of **6** followed by coupling with benzoic acid gave compound **8** which was then cyclized with $POCl_3$ to give intermediate **9**. Oxidation of the methylthio functionality of **9** to the methyl sulfoxide using 1 equiv of *m*-chloroperbenzoic acid (*m*-CPBA) and subsequent displacement with alkyl amines gave compounds **10a–c** (see Table 1).

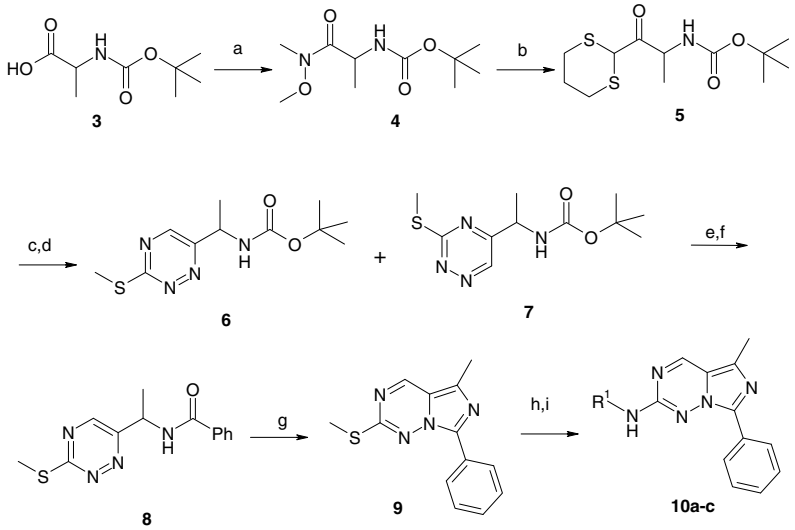


Scheme 2. Reagents and condition: (a) substituted aryl bromide or iodide, (S)-(−)-2,2′-bis(diphenylphosphino)-1,2′-binaphthyl, NaO-*t*-Bu, $Pd_2(dba)_3$, microwave or heating at 80 °C.



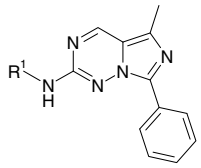
Scheme 3. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, 3 h, 85%; (b) 3,4-5-trimethoxyaniline, cat. TsOH, THF, rt; (c) 4 N HCl in dioxane, MeOH, 30 min; (d) for aryloyl acid: substituted benzoic acid, Et_3N, HATU, DMF; for aryloyl chloride: substituted benzoyl chloride, Et_3N, CH_2Cl_2, −78 °C, 1 h then rt; (e) 6 equiv, 1,2,4-triazole, pyridine, 3 equiv, $POCl_3$, rt, 25–46 h.

In contrast to alkyl amines, direct displacement of methyl sulfoxide group, obtained from mono-oxidation of intermediate **9**, with anilines was not fruitful. Instead, anilino substituted analogs



Scheme 1. Reagents and conditions: (a) MeNHOMe·HCl, CDI, Et_3N, CH_2Cl_2, 98%; (b) 1,3-dithiane, THF, −78 °C; then 2.5 M *n*BuLi, −15 to −25 °C, 2 h, then 4, 1.5 h, −15 °C, 77%; (c) thiosemicarbazide, TsOH, 78 °C, 72 h, 100%; (d) MeI, $CaCO_3$, CH_3CN, 40 °C, 24 h, 17%; (e) 4 N HCl in dioxane, MeOH, 30 min; (f) $PhCO_2H$, Et_3N, HATU, DMF; (g) 6 equiv, 1,2,4-triazole, pyridine, 3 equiv, $POCl_3$, rt, 25–46 h; (h) 1 equiv, mCPBA, CH_2Cl_2, 3 h; (i) R^1NH_2, heat.

Table 1
PLK1 activity for compounds **2**, **11**, **10a–c**.



Compound	R^1	PLK1 enzyme IC_{50}[a] (μM)
2	$-CH_2Ph$	1.5
11	–H	>10
10a	$-CH_2CH_2$-*p*-pyridine	>10
10b	$-CH_2CH_2CH_2$-morpholine	>10
10c	–Cyclohexyl	>10

[a] Values are means of ⩾3 experiments.

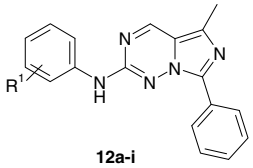
(compounds **12a–i**, see Table 2) were prepared by coupling of aryl halides such as aryl bromides or aryl iodides to 5-methyl-7-phenylimidazo[5,1-*f*][1,2,4]triazin-2-amine (**11**) using standard palladium coupling chemistry (Scheme 2).

In order to explore the SAR around the phenyl substituent at position 7, the synthesis shown in Scheme 3 was developed. Oxidation of thiomethyl derivative **6** followed by treatment with 2,3,4-trimethyoxyaniline yielded compound **13**. Deprotection of the *tert*-butyoxycarbonyl group under acidic conditions and subsequent coupling with either aryl acids or aryl acid chlorides provided compounds **14a–i**. Treatment of **14** with conventional $POCl_3$ cyclization conditions resulted in poor yields (0–20%) of the desired imidazotriazine derivatives. Interestingly, addition of 6 equiv of 1,2,4-triazole significantly improved the $POCl_3$ cyclization reaction to give the imidazotriazines **15a–i** in 30–50% yield.

The PLK1 enzyme inhibitory activity of the compounds prepared is shown below (Tables 1–3). As shown in Table 1, removal of the benzyl group (compound **11**) resulted in significant reduction in the PLK1 activity.[11] Extending the side chain (compounds **10a**, **10b**) or replacing the benzyl group with a cyclohexyl group (compound **10c**) also resulted in reduction of the PLK1 activity.

Interestingly, when the benzyl group at position 2 was replaced by phenyl (compound **12a**), PLK1 potency was maintained (Table 2). Substitution of the phenyl group with various functional groups was tolerated. Compounds with electron donating groups (**12b–d**, **12h**) showed higher PLK1 activity than compounds with electron-withdrawing groups (**12e–g**). Incorporation of the 3,4,5-trimethoxyphenyl substitution (compound **12i**), led to improvement in PLK1 potency by 25-fold over compound **2**.

Table 2
PLK1 activity for compounds **12a–i**.

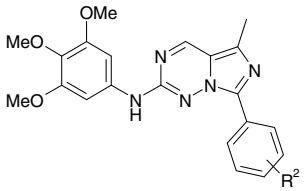


12a-i

Compound	R^1	PLK1 enzyme IC_{50}[a] (μM)
12a	H	1.2[b]
12b	–3-OMe	0.3[b]
12c	–4-OMe	0.68[b]
12d	-4-O-$CH_2CH_2N(Me)_2$	0.38
12e	-3-CF_3	>10
12f	-4-NO_2	2.81
12g	-4-SO_2NH_2	2.74
12h	3,4-DiOMe	0.09
12i	3,4,5-TriOMe	0.04

[a] Values are means of ⩾3 experiments.

Table 3
PLK1 activity for compounds **12i**, **15a–i**.



Compound	R^2	PLK1 enzyme IC_{50}[a] (μM)
12i	H	0.04
15a	2-OMe	5.66
15b	2-NO_2	1.98
15c	2-Br	2.67
15d	3-Br	0.07
15e	3-CF_3	0.03
15f	4-CF_3	1.06
15g	3-C(O)Ph	0.41
15h	3-O-Ph	0.50
15i	4-F	0.08

[a] Values are means of ⩾3 experiments.

Given the enhanced potency observed with the 3,4,5-trimethoxylaniline substituent (**12i**), this group was held constant as we explored the SAR of the aryl ring at the 7-position (Table 3). Compounds with both electron donating (**15a**) and electron withdrawing (**15b–c**) groups at the ortho-position resulted in a drop in PLK1 potency when compared to parent compound (**12i**). Meta-substitution was better tolerated than ortho- and para-substitution as evidenced by 3-Br compound (**15d**) being 38-fold more potent against PLK1 than the 2-Br compound (**15c**), and 3-CF_3 compound (**15e**) being 35-fold more potent than 4-CF_3 compound (**15f**). Compounds with large substituents at the meta-position (**15g–h**) and small substituents at the para-position (**15i**) were also tolerated.

The most active PLK1 inhibitors were evaluated in cellular assays for their ability to inhibit proliferation of a variety of tumor cell lines.[11] As shown in Table 4, compounds **12i**, **15d**, and **15e** were modest inhibitors of proliferation in the tumor cell lines examined.

Compounds **12i** and **15e** were evaluated against a panel of 40 kinases (including PLK3) to assess kinase selectivity. Compounds **12i** and **15e** were found to inhibit PLK3 with an IC_{50} of 0.09 μM and 0.03 μM, respectively, and thus are to be considered dual inhibitors of PLK1 and PLK3. Compound **15e** was at least 10-fold selective for PLK1 and PLK3 versus the other kinases tested. In general, compound **12i** was less selective.

Pharmacokinetic properties of compounds **12i** and **15e** in the mouse were also evaluated (Table 5). Compound **15e** had high clearance (81 mL/min/kg) and modest oral bioavailability (18%*F*), while compound **12i** had moderate clearance (53 mL/min/kg) and a lower oral bioavailability (10%*F*).

To build confidence in the binding mode used in the homology model, an X-ray structure of **12i** bound in CDK2/cyclin A was obtained.[12] As shown in Figure 4, the binding mode of **12i** in the CDK2/cyclin A is consistent with the proposed binding mode in the PLK homology model. The inhibitor hydrogen bonds to the backbone NH and C=O of Leu83 in the CDK2/cyclin A hinge region. It was interesting to note that one of the methoxy groups is within hydrogen bonding distance of the side chain of Asp86 in the CDK2 structure.

In summary, we have discovered a novel series of polo-like kinase inhibitors based on an imidazotriazine template. A PLK1 homology model was employed to identify key areas to explore the structure–activity relationships of the series. Modifications led to an

Table 4
PLK1 enzymatic and anti-proliferative activities for compounds **12i**, **15d**, and **15e**.

Compound	IC_{50}[a] (μM)					
	PLK1 enzyme	RKO (colon)	HCT116 (colon)	H460 (lung)	MCF7 (breast)	PC3 (prostate)
12i	0.04	3.7	5.3	6.5	5.2	12
15d	0.07	7.6	7.8	5.8	11	26
15e	0.03	3.2	3.9	5.4	4.8	7.8

[a] Values are means of ⩾ 3 experiments for PLK1 enzyme assay and values are means of 2–4 experiments for anti-proliferative assays.

Table 5
Pharmacokinetic for compounds **12i** and **15e** in mouse.

Compound	Dose (iv) (mg/kg)	Cl (mL/min/kg)	Vdss (L/kg)	AUCpo (ng h/mL)	%*F*
12i	5[a]	53	1.6	301	10
15e	2.9[b]	81	2.3	264	18

[a] Compound **12i** was formulated in 10% DMSO in solutol solution.
[b] Compound **15e** was formulated in 95:5 intralipid/DMSO for iv dosing and 0.5% HPMC/0.1% Tween 80 for po dosing.

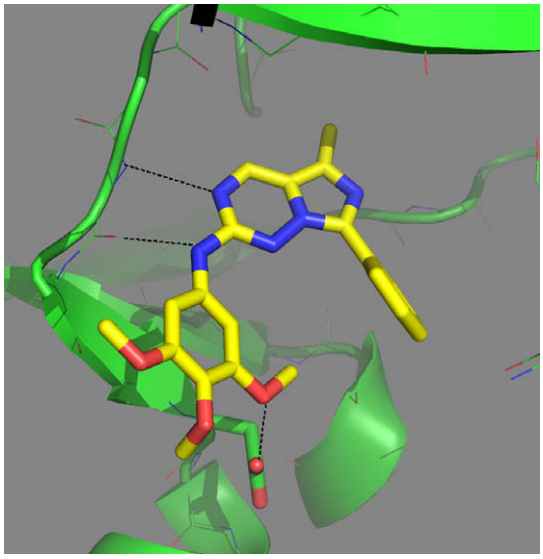


Figure 4. X-ray structure of **12i** bound in CDK2/Cyclin A.

improvement in the PLK1 potency by ~50-fold. In addition, we have developed novel synthetic routes that allow preparation of multiple derivatives to explore the SAR of the imidazo[5,1-*f*][1,2,4]triazin-2-amines series, a novel template for kinase inhibition.

Acknowledgments

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12. X-ray coordinate of **12i** in CDK2/cyclinA has been deposited with the RCSB Protein Data Bank (PDB) database with PDB deposition number of 3EOC. Compound **12i** inhibited CDK2/cyclinA with an IC_{50} of 0.23 μM. CDK2/cyclinA was expressed, purified, and crystallized as previously described.[13] Crystals were cross-linked with 25% glutaraldehyde for 15 min, then soaked with 1 mM compound for 3 days. Prior to data collection, glycerol was added to 25% and the crystals were flash-frozen in liquid nitrogen. Data were collected [99% complete to 3.2 Å resolution (reflections/observations, 33,305/207,344; R_{merge}, 11%)] on an RAXISIV detector and processed with HKL2000.[14] The structure was solved by molecular replacement using Refmac[15] and coordinates 1FVV from the Protein Data Bank. The structure was refined to an R_{factor}/R_{free} of 21/23% at 3.2 Å resolution.
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