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Kinesin spindle protein (KSP) inhibitors. Part 3: Synthesis and evaluation of phenolic 2,4-diaryl-2,5-dihydropyrroles with reduced hERG binding and employment of a phosphate prodrug strategy for aqueous solubility

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Abstract—2,4-Diaryl-2,5-dihydropyrroles have been discovered to be novel, potent and water-soluble inhibitors of KSP, an emerging therapeutic target for the treatment of cancer. A potential concern for these basic KSP inhibitors (**1** and **2**) was hERG binding that can be minimized by incorporation of a potency-enhancing C2 phenol combined with neutral N1 side chains. Aqueous solubility was restored to these, and other, non-basic inhibitors, through a phosphate prodrug strategy.

In the preceding communication, the design, synthesis, and characterization of dihydropyrrole-based inhibitors of KSP (kinesin spindle protein) as novel antimitotics were described.[1] Representative inhibitors **1** and **2** displayed low nanomolar potency against KSP and were shown to induce mitotic arrest in transformed cells (Fig. 1). These inhibitors were promising candidates for further development as intravenous anticancer agents due to their pharmacokinetic profile and aqueous solubility. However, a potential concern for these amide- and urea-based dihydropyrroles **1** and **2** was their ancillary activity against the I_{Kr} potassium channel hERG (human Ether-a-go-go-Related Gene). Blockade

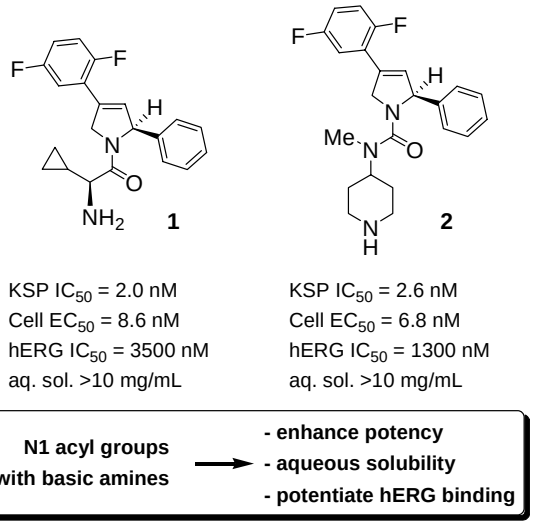

Figure 1. Lead dihydropyrrole KSP inhibitors.

Keywords: KSP; Kinesin spindle protein; Kinesin; hERG; Phosphate prodrug; Cancer; Antimitotic; Dihydropyrrole; Phenol.
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of hERG has been implicated in the drug-induced alteration of cardiac ventricular repolarization that is typically manifested in prolongation of the heart rate-corrected QT interval (QTc).[2] Prolongation of the QTc interval may potentiate cardiac arrhythmia in patients and in rare instances lead to torsades de pointes or sudden death,[3] and therefore part of our optimization efforts were directed to minimize hERG binding.

Structure–activity relationships (SAR) in this series revealed that hERG binding was potentiated by the basic amine in the N1 acyl group. However, deletion or acylation of this amine compromised potency and, to a greater extent, aqueous solubility. An alternative strategy was devised to capitalize on known SAR and X-ray crystallographic information from dihydropyrazole **3**[4] and monastrol[5] wherein a 3′-hydroxyl on the 'eastern' phenyl ring contributes to potency through formation of a hydrogen bond to a backbone carbonyl (Glu118) within the allosteric pocket of KSP (Fig. 2). We hypothesized that for the dihydropyrrole series, similar incorporation of the potency-enhancing phenol would enable replacement of the basic amine at N1 with neutral acyl groups and result in minimization of hERG binding affinity.

An effort to adopt this structural feature into the dihydropyrrole series was undertaken (Scheme 1). Utilizing the three-step racemic route to 2,4-diaryl 2,5-dihydropyrroles[6] described in the preceding communication, intermediate 2,3 dihydropyrrole **5** was examined as a substrate for Heck coupling to a phenol precursor. It was discovered that 2,3-dihydropyrrole **5** undergoes microwave promoted Heck coupling with aryl iodides, and this procedure was optimized for 3-(*tert*-butyldimethylsiloxy)iodobenzene (**6**). The optimized conditions utilized $Pd(OAc)_2$ (0.1 equiv) with Ph_3As (0.2 equiv) as ligand and proceeded for 30 min at 150 °C under microwave irradiation to produce the desired 2,4-diaryl-2,5-dihydropyrrole (**7**) in 56% yield following purification.

The racemic dihydropyrrole (**7**) was resolved into pure enantiomers using chiral chromatography and only the *S*-enantiomer[7] (**8**) was carried forward. Removal of the Boc-protecting group using TFA revealed dihydropyrrole **9** and acylation under conditions described in the preceding communication: N1 amides (**12**) were synthesized by PyBOP coupling, whereas ureas (**13**) were produced either via the N1-acyl imidazolium salt or direct acylation with a carbamoyl chloride. Final deprotection of the phenol was accomplished using K_2CO_3 in DMF to give the phenol-bearing KSP inhibitors (**11**).[8]

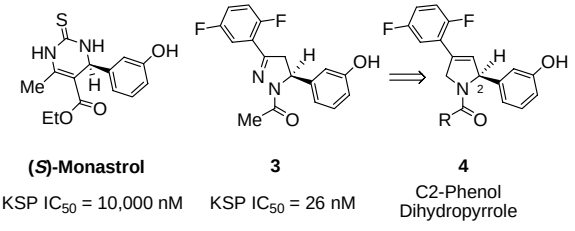
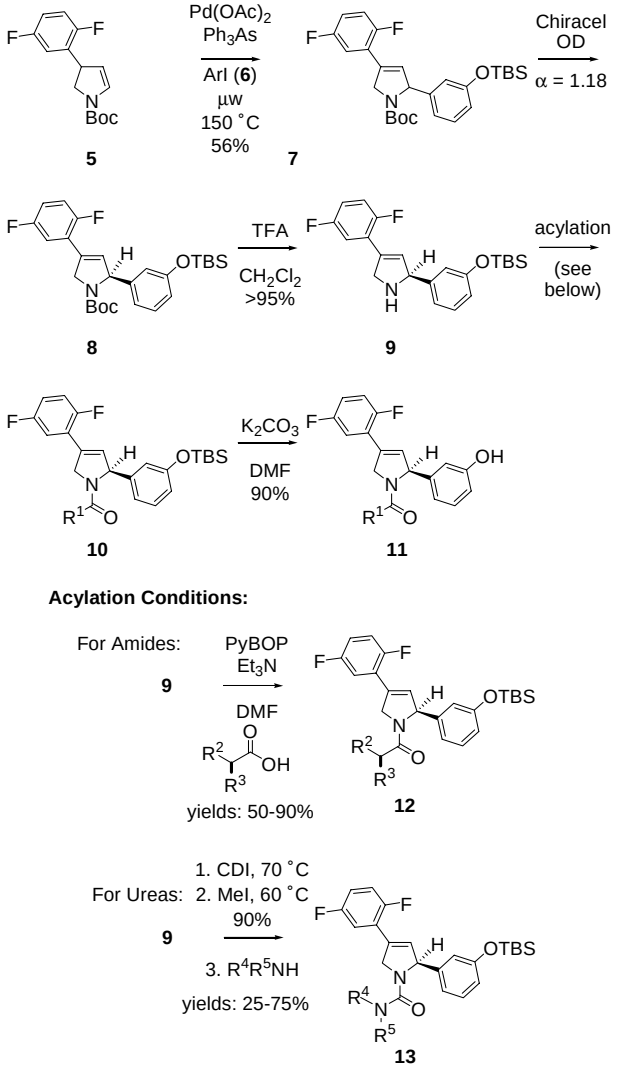


Figure 2. Selected and proposed phenolic KSP inhibitors.

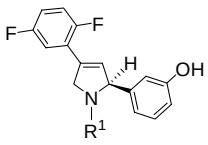


Scheme 1. Synthesis of dihydropyrrole C2-phenols.

Incorporation of the phenol into the amide- and urea-based dihydropyrroles produced a 3- to 5-fold potency enhancement for most inhibitors evaluated (e.g., **1** vs **16**). A representative set of KSP inhibitors is presented (Table 1) along with their potency against KSP,[9] cell potency in A2780 human ovarian carcinoma cells[10], and hERG activity.[11] Combination of the C2-(3′-phenol) with the potency-optimized basic N1-acyl groups afforded highly potent ($IC_{50} < 3$ nM) KSP inhibitors (**15** and **16**) with excellent cell-based activity; however, the hERG binding was only minimally improved by the presence of the phenol alone. The strategy for significant reduction of hERG binding was realized in the combination of the phenol with neutral N1-acyl groups that, together, retained KSP inhibitory activity with $IC_{50} < 10$ nM. KSP inhibitors **17**–**20**, that possessed neutral α-hydroxy amides and ureas, displayed excellent potency and reduced hERG binding. Interestingly, the basic urea **21** did not show significant potency enhancement when combined with the phenol.

Based on their overall profile including diminished hERG activity, phenols **17**–**19** were considered for

Table 1. Properties of selected C2-phenol



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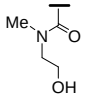
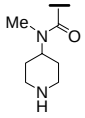
Compound	R^1	KSP IC_{50} (nM)	Cell EC_{50} (nM)	hERG IC_{50} (nM)
14[a]		13	37	7800
15		1.2	3.3	8500
16		0.5	3.0	5600
17		2.9	3.1	18,000
18		1.3	2.6	7800
19		7.0	22	33,000
20		4.0	9.1	15,000
21[a]		5.5	8.5	6600

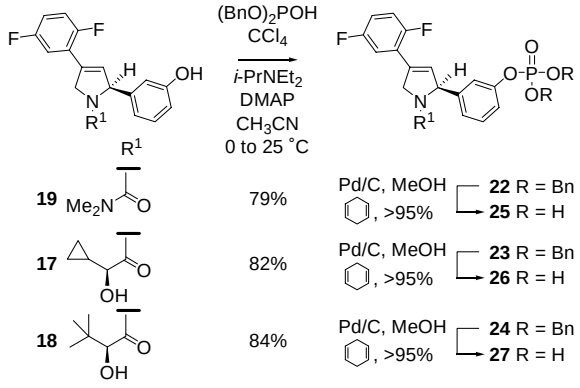
[a] Tested as a racemic mixture.

further characterization along with two additional KSP inhibitors, **28** and **32**.[1] A key limitation to these inhibitors was that, with the absence of the basic amine, aqueous solubility was negligible and additional formulation would be required for use as intravenous agents. It was preferable to avoid the use of vehicles such as Cremaphor© that bear their own side effects and toxicities. To circumvent these potential issues, chemical derivatization to a water-soluble prodrug was examined.

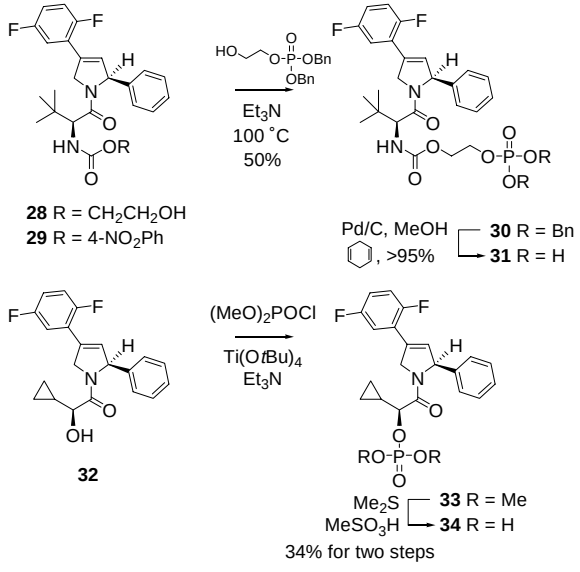
Phosphate prodrugs have been widely used to enhance the aqueous solubility of parent drugs, and this strategy has been adopted for other clinical anticancer agents including etoposide, pancratistatin and taxol.[12] The anionic charge imparted by the phosphate at nearly all physiological pH values dramatically increases the polarity and aqueous solubility over the parent molecule. Upon dosing in vivo, these phosphates are expected to undergo rapid hydrolysis by non-specific phosphatases and to produce the parent drug with bioequivalence and non-toxic inorganic phosphate.

Typically the phosphate moiety is appended to an available hydroxyl functional group in a parent molecule, and KSP inhibitors **17**–**19**, **28** and **32** were ideally suited for this strategy. Interestingly, three distinct synthetic strategies were required to access phosphates of a phenol, primary alcohol and secondary alcohol, respectively. For the phenols (**17**–**19**, Scheme 2), in situ generation of dibenzylchlorophosphate[13] gave in high yield dibenzylphosphate esters (**22**–**24**). As reported, this procedure is highly chemoselective for phenols over aliphatic alcohols. Transfer hydrogenation of the benzyl groups yielded the desired phosphate prodrugs (**25**–**27**) in >95% yield and purity.

For the primary alcohol (**28**), the dibenzylphosphate was synthesized in a convergent manner (Scheme 3). Phosphorylated ethylene glycol was coupled to the 4-nitrophenylcarbamate (**29**) at 100 °C in neat Et_3N. Transfer hydrogenation produced the desired prodrug **31**. In the final case, the hindered secondary alcohol (**32**) was phosphorylated by chlorodimethylphosphate in the presence of catalytic $Ti(OtBu)_4$.[14] Deprotection was effected by methanesulfonic acid in dimethylsulfide to give the prodrug **34**. Importantly, the presence of a phosphate rendered these compounds inactive (phenols



Scheme 2. Synthesis of phenolic phosphate prodrugs.



Scheme 3. Synthesis of aliphatic phosphate prodrugs.

Table 2. Properties of selected phenols and prodrugs

Drug[a]		Clearance (mL/min/kg)	$t_{1/2}$ (h)	Prodrug[a]	$t_{1/2}$ (h)	AUC of parent (%)[b]
19	Dog	13	1.5	**25**	0.25	100
	Rat	68	0.3	**25**	0.15	100
28	Dog	11	2.8	**31**	<0.1	38
	Rat	68	0.3	**31**	<0.1	57

[a] Dosed in DMSO at 0.4 mpk iv to dog and at 1 mpk iv to rat.
[b] Ratio of AUC's for parent from prodrug dosing and direct parent dosing.

>1 μM) or less active (alcohols >100 nM) towards KSP in the enzymatic assay, and thus the phosphate prodrug itself should not contribute to the antiproliferative effect. In all cases, the aqueous solubility of these inhibitors was dramatically improved by the phosphate group from <1 to >20 mg/mL at pH 7.

An important consideration for the use of phosphate prodrugs is their rate of conversion to parent in vivo, and these varied significantly for the prodrugs studied (Table 2). The dog and rat pharmacokinetic profiles for the parent KSP inhibitors **19** and **28** and their corresponding prodrugs **25** and **31**, respectively, are shown in detail. The key parameter is a comparison of AUC derived from direct dosing of the parent and the AUC of parent achieved by prodrug dosing. When the phenolic phosphate **25** was dosed to rats and dogs, it provided approximately 100% of the AUC seen with direct dosing of the parent (iv), indicative of complete prodrug-to-parent conversion. The prodrugs for both aliphatic alcohols (**31** and **34**), however, appeared to also undergo clearance mechanism(s) other than conversion to parent; for **31** the percent AUC of parent achieved was 57% in rats and 38% in dogs when the prodrug was dosed. Not surprisingly, the hindered secondary phosphate **34** produced little parent and showed poor conversion in other in vivo studies. In general, the phenolic phosphates appeared to be well-behaved prodrugs showing bioequivalence to their corresponding parent drug.

In conclusion, 2,5-dihydropyrroles consisting of a 3′-phenol at C2 were synthesized and found to display enhanced potency against the mitotic kinesin KSP versus their C2-phenyl counterparts. In combination with preferred neutral N1 acyl groups, these phenols showed an improved hERG binding profile, but limited aqueous solubility. A phosphate prodrug strategy was employed that engendered >40 mg/mL aqueous solubility to the lead phenols, and these phenolic phosphates more readily converted to parent than did the corresponding aliphatic phosphates. These potent, water-soluble KSP inhibitor prodrugs are suitable for iv administration and may exhibit safer ancillary profiles due to their reduced affinity for hERG.

Acknowledgments

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References and notes

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7. Chiracel OD column, 85% hexanes/10% MeOH/5% EtOH, $\alpha = 1.18$, *S* was second to elute.
8. All compounds were characterized by ^{1}H NMR and high resolution mass spectrometry. For detailed experimental procedures, see WO03105855 and WO04111193 (prodrugs).
9. KSP inhibitory activity was measured using a standard ATPase assay. IC_{50} values are reported as averages of at least two determinations; standard deviations are ±25–50%.
10. Mitotic arrest was measured by antibody-based detection of phosphonucleolin, an M-phase marker protein. Values are reported as averages of at least two determinations; standard deviations are ±25–50%. See Ref. 1 for conditions.
11. The hERG IC_{50} values are reported as averages of at least two determinations and were acquired by binding competition experiments using membrane preparations from human embryonic kidney cells that constitutively express hERG.
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