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Potent Quinoxaline-Based Inhibitors of PDGF Receptor Tyrosine Kinase Activity. Part 1: SAR Exploration and Effective Bioisosteric Replacement of a Phenyl Substituent☆

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Abstract—Novel substituted 2-anilino- and 2-cycloalkylaminoquinoxalines have been found to be useful and selective inhibitors of PDGF-R autophosphorylation. Replacement of an anilino-substituent with substituted cyclohexylamino- or norbornylamino substituents led to significant improvements in the pharmacokinetic profile of these analogues.

Percutaneous transluminal coronary angioplasty (PTCA) is a highly successful surgical procedure used to clear blocked coronary arteries. The process is relatively non-invasive and easily performed; it is estimated that more than one million procedures are performed annually worldwide. The incidence of acute side effects is very low and patients recover rapidly. However, PTCA leads to a process termed restenosis that involves the migration, proliferation, matrix formation and remodeling associated with the initial injury. This process can lead to significant coronary artery blockage over a period of up to 6 months in 25–40% of all patients.

New surgical technologies including the placement of metallic stents have been employed in attempts to limit restenosis.[2] Despite the successes in stent technology for acute restensosis, the rate of long-term restenosis remains relatively constant. A host of clinical trials over the last 15 years with a large variety of pharmacological agents have also failed to impact the rate of restenosis significantly. However, recent success in lowering the rate of restenosis has been reported using drug-coated metallic stents.

The key role of platelet derived growth factor (PDGF) and its receptor (PDGF-R) in the process leading to long-term restenosis after PTCA has been investigated in depth.[3] The growth factor and its receptor have also been implicated in wound healing, tumorigenesis, angiogenesis and atherosclerosis. Our early discovery efforts to find a selective inhibitor of PDGF-R autophosphorylation[4] resulted in the identification of a now well-studied prototype, RPR101511A (Table 1), which was ultimately evaluated in the Yucatan mini-pig model.[5] The positive activity seen in this rigorous model of restenosis gave us significant confidence in proceeding with our optimization program. Our objectives were thus: (1) to improve in vitro activity, (2) to improve pharmacokinetic profile, (3) to maintain selectivity and (4) to expand the SAR of the series.

Herein, we report our first written communication on these optimization efforts and we present an interesting SAR study regarding the activity of this class of ATP-competitive PDGF-R inhibitors.

Hetereoatom linked molecules, generically described as **1** in Figure 1, were initially targeted as PDGF receptor tyrosine kinase inhibitors. It was thought that the ease of synthesis and the potential for a rapid exploration of SAR would be profitable. The general methods of preparation for these compounds have already been reported in a series of published PCT applications.[6]

☆This work was presented in part at the conferences shown in ref 1.

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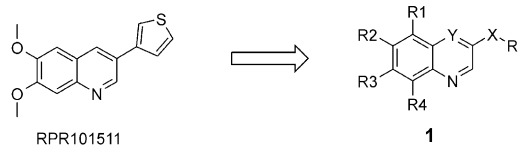
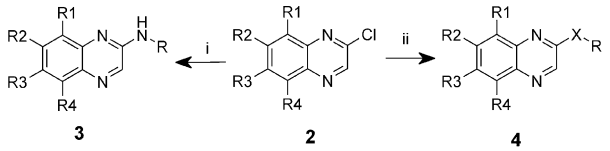
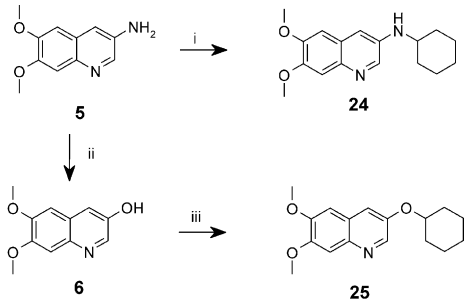


Figure 1. RPR101511 and hetereoatom linked analogues.



Scheme 1. Synthesis of quinoxaline analogues: (i) $RNH_2/180\,^{\circ}C$; (ii) RONa/THF.

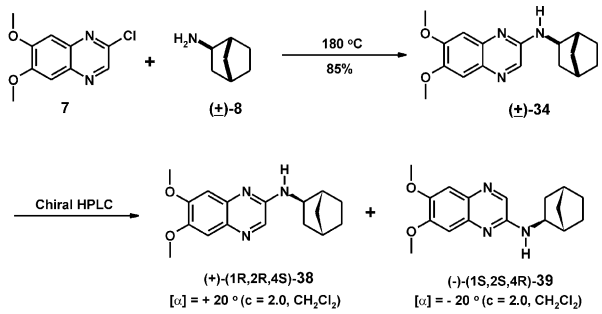


Scheme 2. Synthesis of quinoline analogues: (i) cyclohexanone, $NaBH_3(CN)$; (ii) $NaNO_2/HCl$; (iii) cyclohexanol/DEAD/Ph_3P.

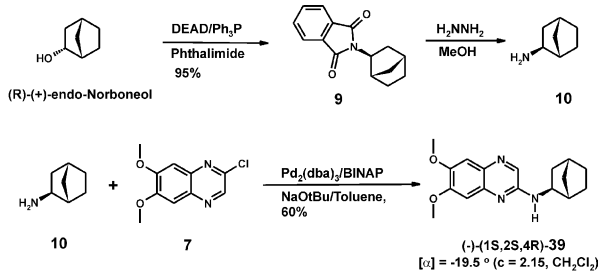
Generally, the amino-substituted quinoxaline derivatives **3** were prepared by heating a >5-fold excess of the amine or aniline with the appropriate 2-chloroquinoxaline **2** for 3–12 h at 180 °C (Scheme 1). These reaction conditions limited our ability to prepare a diverse array of analogues from sensitive or precious amines directly. Significant amounts of des-methyl impurities were often isolated in the coupling of less reactive amines with 2-chloro-6,7-dimethoxyquinoxaline. The preparation of the oxy and thio derivatives **4** (X = O, S) from the corresponding alkali salts was straightforward. Such reactions proceeded at much lower temperature and were complementary to the fusion reaction for the synthesis of aminoquinoxalines **3**.

The preparation of the quinoline derivative, **24**, was carried out by reductive amination with 3-amino-6,7-dimethoxyquinoline **5**[b] and the appropriate ketone. The oxy-substituted quinoline **25** was prepared via Mitsunobu reaction with 6,7-dimethoxy-3-hydroxyquinoline **6** and the corresponding alcohol (Schemes 2 and 3).

The racemic *exo*-norbonyl derivative **34**, prepared by the fusion reaction of compounds **7** and **8**, was resolved by chiral HPLC to afford two single enantiomers **38** and **39**. An asymmetric synthesis was conducted to confirm the absolute stereochemical assignments as outlined in Scheme 4. The preparation begins with the commercially available (+)-(*R*)-endo-norboneol; Mitsunobu



Scheme 3. Synthesis and resolution of norbonyl analogues.



Scheme 4. Asymmetric synthesis of the norbonyl analogue.

inversion using pthalimide followed by hydrazine-mediated liberation of the amine provided **10**. We then applied the palladium-mediated coupling conditions of Buchwald[10] in order to conserve the precious chiral amine **10**. Optical rotation studies confirmed that the levorototory isomer is **39**.

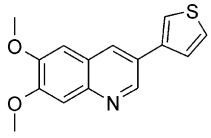
Our early prototype PDGF-R inhibitor, RPR101511, has an IC_{50} of 461 nM in an ELISA-based assay for PDGF-R autophosphorylation. Simple insertion of a nitrogen between the two aromatic rings leads to a nearly 2-log improvement in activity (**17** vs RPR101511). For comparison, the des-dimethoxy derivative, **11**, is 1 log less potent than RPR101511. However, a single methoxyl substitution can improve potency by up to 100-fold depending on the position of substitution (compare **13**, **14**, **15**, **16** vs **11**). It is clear that substitutions at the 5 and 8 positions of quinoxaline are less tolerated than those at the 6 and 7 positions (**15**, **16** vs **13**, **14**). The fact that the dimethyl substituted anilinoquinoxaline **12** is 60-fold less potent than the dimethoxyl analogue **17** seems to suggest that the two oxygen atoms might be involved in hydrogen bonding and that this region of the quinoxaline could be used as a handle for chemical manipulations in order to change the chemical and physical properties of target molecules. Unfortunately, these anilino analogues suffered a similar pharmacokinetic profile to RPR101511 in that they induced p450 enzymes (in rat) that led, over the course of several days dosing, to a greatly diminished exposure via oral administration as discussed further in the following paper.

The first significant breakthrough towards an improvement in PK profile was realized when compound **22** was evaluated side-by-side with RPR101511 in an in vitro assay of p450 upregulation (data not shown). It was determined that **22** did not induce upregulation.

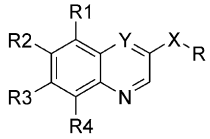
Moreover, single-dose po administration over 4 days demonstrated that exposure levels were maintained between days 1 and 4, albeit with low exposure. These positive results and the fact that **22** was only 4-fold more potent than RPR101511 led us to explore further the SAR of substituted cycloalkyl derivatives. The highlights of some of our results are presented in Table 1.

It can be seen that the cyclohexylamino quinoline analogue **24** was slightly more potent than quinoxaline **22**. Interestingly, the oxo quinoline derivative **25** was ~5-fold less potent than **22** and **24** but the cyclohexyloxy-quinoxaline, **23**, was 2-fold more potent. Going further it can be seen that cyclopentyl is nearly equivalent to cyclohexyl for the oxo and amino linkers (**26** vs **22** and **27** vs **23**). Addition of one carbon atom (**33**) significantly

Table 1. PDGF-R activity



RPR101511



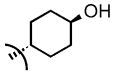
Compd	R1	R2	R3	R4	X	Y	R	PDGF-R (IC_{50}, μM)
RPR101511								0.461
11	H	H	H	H	NH	N	Ph	5.1
12	H	Me	Me	H	NH	N	Ph	0.34
13	MeO	H	H	H	NH	N	Ph	11% @ 1 μM
14	H	H	H	MeO	NH	N	Ph	5% @ 1 μM
15	H	H	MeO	H	NH	N	Ph	1.6
16	H	MeO	H	H	NH	N	Ph	0.042
17	H	MeO	MeO	H	NH	N	Ph	0.006
18	H	MeO	MeO	H	NMe	N	Ph	0% @ 1 μM
19	H	MeO	MeO	H	NH	N	3Cl–Ph	0.004
20	H	MeO	MeO	H	NH	N	4Cl–Ph	8% @ 1 μM
21	H	MeO	MeO	H	NH	N	Bn	0.092
22	H	MeO	MeO	H	NH	N	Cyclohexyl	0.124
23	H	MeO	MeO	H	O	N	Cyclohexyl	0.065
24	H	MeO	MeO	H	NH	CH	Cyclohexyl	0.096
25	H	MeO	MeO	H	O	CH	Cyclohexyl	0.601
26	H	MeO	MeO	H	NH	N	Cyclopentyl	0.128
27	H	MeO	MeO	H	O	N	Cyclopentyl	0.159
28	H	MeO	MeO	H	S	N	Cyclohexyl	0% @ 1 μM
29	H	MeO	MeO	H	S	N	Cyclopentyl	0.226
30	H	MeO	MeO	H	NH	N	3-Methylcyclohexyl	0.041
31	H	MeO	MeO	H	O	N	4-Ethylcyclohexyl	16% @ 1 μM
32	H	MeO	MeO	H	NH	N		0.076
33	H	MeO	MeO	H	NH	N		12% @ 1 μM
34	H	MeO	MeO	H	O	N	 (±)	0.025
35	H	MeO	MeO	H	O	N	 (±)	0.265
36	H	MeO	MeO	H	NH	N	 (±)	0.020
37	H	MeO	MeO	H	NH	N	 (±)	0.879
38	H	MeO	MeO	H	NH	N	 (+)	0.015
39	H	MeO	MeO	H	NH	N	 (-)	0.742

Table 2. Comparison of inhibition of PDGF-R autophos phorylation and mitogenesis in human aortic smooth muscle cells

Compd	PDGF-R (IC_{50}, μM)	HASMC (IC_{50}, μM)	Ratio activity mito./enzyme
32	0.076	0.353	5
34	0.025	0.395	15
38	0.015	0.061	4
39	0.742	0.585	0.8

decreases activity. Exchange of the amine or oxo linker for sulfur also leads to a dramatic loss in activity for the cyclohexyl analogue **28**, whereas a loss of only 2-fold or less is seen for the thio cyclopentyl derivative (**29**). This set of results underscores the significant steric constraints imposed by the lipophilic binding pocket adjacent to the ATP-binding domain and are consistent with the steric restrictions seen in our earliest studies.[7] This binding domain is often cited as being crucial to kinase selectivity and potency. Insensitivity to replacing the NH-linker by a sulfur or oxygen also implies that there is no absolute requirement for a hydrogen bond donor.

To this last point, replacement of NH with a NMe-linker, (**18** vs **17**), leads to significant loss of activity in the aryl series. We suggest that this is due to steric constraints of the lipophillic binding domain and/or conformational issues with the orientation of the aryl or cycloalkyl lipophillic substituent rather than loss of a significant H-bond between the NH-linker and the protein backbone.[8] We found that the NH-linked analogues generally exhibited superior physicochemical profiles relative to the oxo or thia derivatives although we continued to exploit all of these for our SAR studies.

The next significant advance in our studies came with the observation that the racemic oxy-linked norbornyl analogue **34** exhibited 25 nM activity, thus recovering much of the loss seen on going from anilino analogues (**17**) to simple cycloalkyls (**22**, **26**). Clearly the *exo* orientation is preferred over endo (**34** vs **35**). Simple replacement of the oxygen with an NH actually leads to no change or a slight loss in activity for the exo and endo racemic mixtures (**34** vs **36** and **35** vs **37**). *A 50-fold preference for the* exo *derivative* **38** *versus its enantiomer* **39** *is worthy of note*. When evaluated for in-cell inhibition of PDGF-stimulated mitogenesis these two analogues demonstrate a 10-fold separation in IC_{50} values (Table 2). Generally, our most potent compounds in the ELISA assay tend to be the most active in the mitogenesis assay. In situ autophosphorylation can be used to confirm that mitogenesis activity is indeed associated with inhibition of PDGF autophosphorylation.

The next active hit from the series was identified from a small library of compounds prepared via reductive amination with a series of 3-aminoquinoline derivatives and a number of aldehydes and ketones (data not shown). Subsequent coupling of 3-methylcyclohexylamine with 2-chloro-6,7-dimethoxyquinoxaline provided the target as a mixture of *cis*- and *trans*-isomers, **30**, which was 3-fold more potent than the parent compound **22**. In contrast, substitution with an ethyl group at the 4-position of cyclohexyl ring resulted in significant loss of activities (**31** vs **22**).

Cyclohexane has long been considered a bioisosteric equivalent for a phenyl group mainly based on size and lipophillic nature. In our case, replacement of the phenyl with cyclohexane results in a 20-fold loss in activity and a significant change in PK profile. A similar improvement in PK profile was reported previously by Tucker et al.[9] in a study of thrombin inhibitors. However, in their case the improvement in exposure did not provide a compound with improved efficacy due to strong protein binding. For our purposes, it appears that both norbornane and 3-methylcyclohexane are equivalent bioisosteres of phenyl for PDGF-R. Potentially significant advantages are readily apparent with these bioisosteres of benzene: (1) greater number of sites for substitution allowing for greater diversity; (2) crystallinity parameters can be significantly changed (interrupt π–π stacking) possibly leading to improved dissolution; (3) a significant reduction in p450 upregulation leading to reduced exposures, and (4) potential for reduced p450 enzyme interactions due to the non-aromatic pharmacophore.

In conclusion, highly optimized PDGF-R tyrosine kinase inhibitors have been identified. The initial anilino-leads suffered from significant issues with bioavailability and induction of p450 enzymes. However, optimized analogues like **32** (RPR127963) have been shown to have superior profiles making them useful compounds for further in vivo profiling as presented in the accompanying paper. Simple bioisosteric replacements for the aryl group were shown to have a significant positive impact on PK properties; a principle which should be generally applicable to other families of inhibitors.

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