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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3097–3100

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent Quinoxaline-Based Inhibitors of PDGF Receptor Tyrosine Kinase Activity. Part 2: The Synthesis and Biological Activities of RPR127963 an Orally Bioavailable Inhibitor☆

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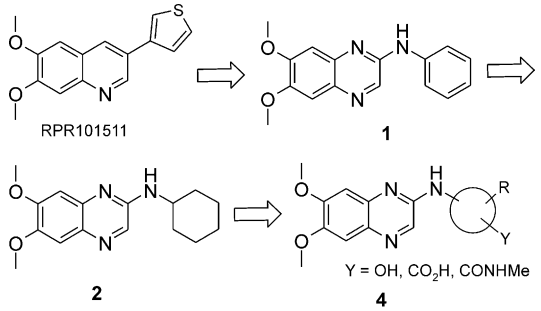
Received 14 April 2003; accepted 20 May 2003

Abstract—RPR127963 demonstrates an excellent pharmacokinetic profile in several species and was found to be efficacious in the prevention of restenosis in a Yucatan mini-pig model upon oral administration of 1–5 mg/kg. The in vitro selectivity profile and SAR of the highly optimized PDGF-R tyrosine kinase inhibitor are highlighted.

Overexpression of PDGF and PDGF-R have been observed in patients with several diseases such as restenosis, certain types of cancers, atherosclerosis, lung fibrosis, kidney fibrosis, liver cirrosis, palmar fibrosis as well as rheumatoid arthritis.[2] Therefore, PDGF receptor tyrosine kinase inhibitors may provide new treatments of many chronic diseases. For example, the recent approval of Gleevec (CGP57148B or STI-571) for the treatment of GI cancers is partially due to its activity against PDGF and c-kit receptor tyrosine kinases, in addition to its original indication for CML related to its activity against abl tyrosine kinase.[3] The challenge for us was to identify orally bioavailable compounds with sustained plasma level of compounds upon chronic oral administrations. In the preceeding paper[4] we disclosed two novel series of hetereoatom linked quinoxaline and quinoline derivatives as potent PDGF receptor tyrosine kinase inhibitors. In this paper we highlight the activity and selectivity profile of RPR127963 that has been shown to have an excellent pharmacokinetic profile in several species. RPR127963 successfully demonstrated efficacy in a Yucatan mini-pig model of restenosis[5] and was ultimately was selected for advanced preclinical studies.

As elucidated in the preceeding paper, aniline analogues such as **1** were about 100-fold more potent than RPR101511A in both the ELISA and Mitogenesis assays. Unfortunately, these analogues suffered a similar pharmacokinetic profile to RPR101511 in that they induced p450 enzymes (in rat) which led over the course of several days dosing to greatly diminished oral exposure.



However, compound **2** less potent than **1** did not induce upregulation and more importantly was observed with

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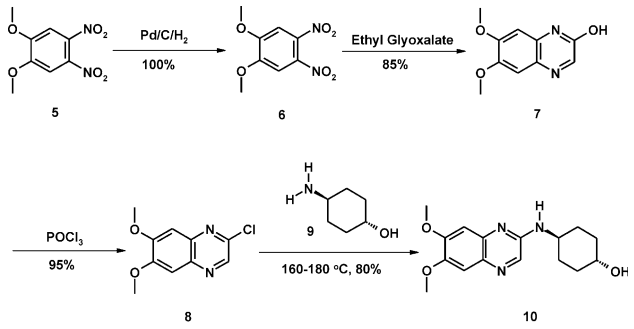
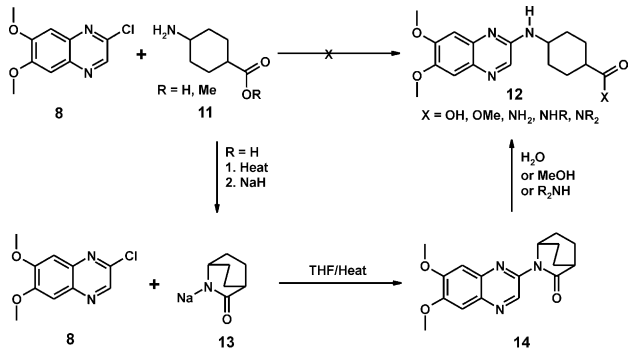
doi:10.1016/S0960-894X(03)00655-3



Scheme 1. Synthesis of RPR127963.



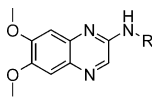
Scheme 2. Synthesis of functionalized quinoxaline analogues.

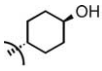
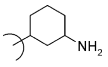
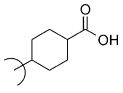
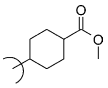
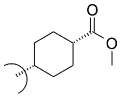
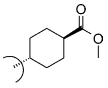
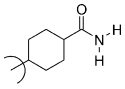
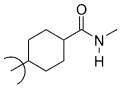
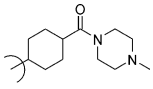
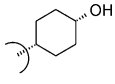
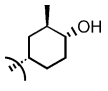
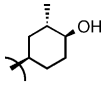
the same exposure levels in plasma between days 1 and 4 upon oral administration once daily over a period of 4 days, albeit with low exposure as shown in Table 2. With these positive results and the fact that **2** was only 4-fold more potent than RPR101511 led us to explore further the SAR of substituted cycloalkyl derivatives. Our designing strategies were to incorporate functional groups Y for exploring the potential improvement of physicochemical properties of the inhibitors as well as the enhancement of potency.

The general methods of preparation for these compounds were exemplified in Scheme 1. Compounds were typically prepared by heating a > 5-fold excess of the appropriate amines with the 2-chloroquinoxaline for 3–12 h at 180 °C. For the synthesis of RPR127963 (compound **10**) this method proved to be fairly robust and all the starting materials were readily available and inexpensive. RPR127963 has been prepared at a very large scale (kg).

However, the thermal coupling reaction failed with cyclohexylamines that are substituted with functional groups such as acids or esters (Scheme 2). This problem was circumvented by designing an alternative approach. Thus, 4-amino-cyclohexanyl carboxylic acid was cyclized first to give the corresponding lactam.[6] The sodium salt of such lactam, **13**, coupled smoothly in THF with 2-chloro-6,7-dimethoxyl-quinoxaline to a versatile intermediate **14**. Treatment of intermediate **14** with various nucleophiles provided aminoquinoxaline derivatives **12** that possess acid, ester, amide functional groups (**17**, **18**, **21**, **22**, and **23**). Ester **18** was separated into *cis*-isomer **19** and *trans*-isomer **20**. Compounds **25**,

Table 1. PDGF-R Activity



Compd	R	PDGF-R (IC_{50}, μM)
RPR101511		0.461
1		0.006
2		0.124
10		0.076
14		14% @ 10 μM
15[a]		0.041
16[a]		−9% @ 1μM
17[a]		15% @ 1μM
18[a]		0.049
19		0.028
20		0.345
21[a]		0.93
22[a]		3.1
23[a]		10% @ 1μM
24		0.068
25		5% @ 1μM
26		0.018
27		0.024

[a]Mixture of *cis*- and *trans*-isomers.

26 and **27** were made from the corresponding amino alcohols.[7]

The biological data for these analogues are included in Table 1. The replacement of the methyl group of compound **15** with a basic amino group dramatically decreased the inhibitory activity against PDGF-R tyrosine kinase (**16** vs **15**). For unknown reasons, a basic group is not tolerated at this region. This is a general trend with other compounds (not shown) that also possess basic functional groups at the 3- or 4-position of the cyclohexyl ring. The substitution at the 4-position with a carboxylic acid group also resulted in the loss of activity (**17** vs **2**) while the methyl ester substitution at the same position slightly increased the potency (**18** vs **2**). Although the *cis*-isomer **19** was 10-fold more active than the *trans*-isomer **20** in the ELISA assay, neither these esters (**18**, **19** and **20**) nor the acid (**17**) showed any significant activity in the cellular PDGF-induced mitogenesis assay. Such results were not really surprising. These esters were probably hydrolyzed to the acid by cellular esterases under the functional assay conditions. Unfortunately, more stable amide analogues **21**, **22** and **23** are much less active against PDGF-R tyrosine kinase with micromolar potency at best.

Our breakthrough was achieved with hydroxylation of the saturated rings. Interestingly, we discovered that a hydroxy group substitution to the cyclohexyl and norbonyl rings maintains the potency (**10**, **24**, **26** and **27** vs **2**). *trans*-Isomer **10** and *cis*-isomer **24** were equipotent in the ELISA assay. More importantly, compound **10** was observed to have a greatly improved PK profiles in a rat PK study as shown in Table 2. Gratifyingly, oral administration of the most potent isomers in both the substituted cyclohexyl and norbonyl series (**26**, and **27**) in rats resulted in an overall exposure that was significantly improved relative to compound **10**. Ultimately, RPR127963 was selected for evaluation in our Yucatan mini-pig model of restenosis despite the improved in vitro potency and in vivo ADME properties of **26** and **27** due to the relative ease of preparing the large quantities of material required for a chronic study.

Table 2. Bioavailability and p450 Induction

Compd	Day 1 (%)	Day 4 (%)	P450 upregulation (in vitro, rat)
RPR101511	49	21	Yes
2	17	16	No
10	82	72	No

Table 3. Comparison of inhibition of PDGF-R autophosphorylation and mitogenesis in human aortic smooth muscle cells

Compd	PDGF-R (IC_{50}, μM)	HASMC (IC_{50}, μM)	Ratio activity mitogenesis/enzyme
10	0.076	0.353	5
25	5% @ 1 μM	>10.0	—
26	0.018	0.134	7
27	0.024	0.164	7

The cellular functional activity of a certain number of analogues is presented in Table 3. The most potent compounds in our ELISA assay tended to be the most active in the mitogenesis assay. Compound **25** demonstrated only very weak activity in our PDGF-R assay and was not significantly active in the mitogenesis assay.

The selectivity profile and functional activities of RPR127963 are presented in Tables 4 and 5. In most cases we evaluated direct enzyme inhibition. However, for some targets (i.e., HGF) functional activity was measured. The only significant activity discovered for RPR127963 beyond PDGF-R was for EGF-R and $p56^{lck}$. From our experience it is anticipated that the low micromolar level of in vitro activity against these particular kinases would not translate into significant functional activity. We also measured the inhibition of PDGF-BB or PDGF-AA stimulation of mitogenesis and inhibition of PDGF-BB stimulated chemotaxis and collagen production. Nearly identical IC_{50}s of about 300 nM were found. These results coupled with the lack of anti-mitogenic activity of compounds like **25** (Table 3) support our contention that RPR127963 is a very selective pharmacological tool.

As a consequence of the excellent in vitro and in-cell profile as well as an excellent ADME profile in rat we chose to evaluate the PK profile of RPR127963 further. Its superior PK profiles were observed in many species including dogs and mini-pigs. RPR127963 was found to have a linear pharmacokinetic profile in several PK studies in Yucatan min-pigs with an oral bioavailability of 100% and a very long half-life. Subsequently, RPR127963 was evaluated in the Yucatan mini-pig model of restenosis and it was found to be orally active in two separate trials (PTCA and implanted stent) at doses of 1–5 mg/kg/day for 28 days as determined by

Table 4. Selectivity profiles of RPR127963

Kinase	IC_{50} or I% (*N*) (μM)
PDGFr	0.076±0.013 (7)
EGFr	3.7
CSF-1r	2% @ 30
VEGF	0% @ 30 (3)
Insulin r (glucose transporter)	33±5 (4)
IGF (mitogenesis)	≫10 (3)
SYK	54% @ 10
ZAP70	0% @ 10
JAK2	>50
JAK3	>50
JNK	>50
LCK	1.4±0.1 (3)
HGFr (chemotaxis)	17% @ 30
PKA	3% @ 100
PKC	7% @ 100

Table 5. Additional functional activity of RPR127963

Assay	IC_{50} or I% (*N*) (μM)
Mitogenesis (PDGF-BB)	0.353±0.071 (11)
Mitogenesis (PDGF-AA)	0.299±0.055 (3)
Chemotaxis	0.292±0.075 (3)
Collagen production	0.389±0.144 (3)

angiographic, intravascular ultrasound and histological criteria. The experimental details and results of these studies will be published elsewhere.

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. The optimized analogues have been shown to have superior profiles making them useful compounds for further in vivo profiling. The oral activity of compound **10** at a relatively low dose and a chronic administration in animal models represent a major improvement over the prototype compound RPR101511. The results of additional studies of RPR127963 will be reported in due course.

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7. The synthesis of the corresponding amino alcohols will be published later.