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Structure–activity relationships and hepatic safety risks of thiazole agonists of the thrombopoietin receptor

Amy S. Antipas, Laura C. Blumberg [†], William H. Brissette, Matthew F. Brown, Jeffrey M. Casavant, Jonathan L. Doty, James Driscoll [‡], Thomas M. Harris, Christopher S. Jones, Sandra P. McCurdy, Eric McElroy, Mark Mitton-Fry [*], Michael J. Munchhof, David A. Reim [§], Lawrence A. Reiter, Sharon L. Ripp, Andrei Shavnya, Marc I. Smeets, Kristen A. Trevena

Pfizer Worldwide Research and Development, Groton, CT 06340, USA

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ABSTRACT

5-F substitution of an aminothiazole moiety within a series of thrombopoietin receptor agonists leads to potent agents with an improved hepatic safety profile in rodent toxicology studies.

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The term thrombocytopenia denotes a condition in which platelet levels drop to below 50,000/μL. It occurs in immune thrombocytopenia purpura (ITP), in association with cancer chemotherapy, and in instances of impaired liver function.[1] The potential for spontaneous bleeding can arise in all of these conditions. Until recently the only available treatment was platelet infusion, but the recent approvals of the oral drug eltrombopag[2] and the injectable romiplostim[2,3] have offered promising new options.

The isonipecotic acid derivative **1** (Fig. 1) was recently reported as an orally bioavailable agonist of the human thrombopoietin (TPO) receptor.[4] Although this compound had promising potency and pharmacokinetic properties, the presence of a 5-unsubstituted-2-aminothiazole was of significant concern, owing to the propensity for such thiazoles to undergo metabolic activation to generate potentially hepatotoxic species.[5] As a result, the biotransformation of **1** and its hydrolysis product **2** were examined. Although **1** did not produce any detectable glutathione adducts, the treatment of human liver microsomes with **2** led to the formation of such adducts, indicating that oxidative bioactivation had occurred.[6]

We now report that toxicological studies of **1** yielded additional potential evidence of hepatic damage. When rats were subjected to single oral doses ranging from 50 to 500 mg/kg, dramatic increases in both AST and ALT were observed at all dose levels (Table 1). Although histopathology revealed no correlated liver changes, the brief exposure produced by a single dose may not have been sufficient to induce any such changes. Additionally, when **1** was dosed orally at 100 mg/kg in transgenic mice expressing the human TPO receptor,[7] mild elevations in AST were observed, although ALT levels were unchanged. These and other studies revealing acute toxicity at 250 mg/kg in rats indicated that **1** did not have a safety profile that was commensurate with further development.

While these studies did not establish a causal link between the 5-unsubstituted-2-aminothiazole moiety and the observed hepatotoxicity, we nevertheless sought to mitigate this possibility in further analogs by eliminating this structural feature, either by adding a substituent at C-5 of the thiazole or by altering the heterocycle itself. In one example from the realm of non-steroidal anti-inflammatory agents, incorporation of a substituent at C-5 of a 5-unsubstituted-2-aminothiazole converted the hepatotoxic sudoxicam to the successful and non-hepatotoxic drug meloxicam.[8]

Accordingly, we introduced a 5-F group onto the thiazole ring yielding **3**. However, this analog was significantly less potent than **1** in the BaF_3 reporter assay, which measures thrombopoietin receptor agonist activity (Table 2).[9] Previous related medicinal chemistry efforts had revealed that co-planarity of the pendant phenyl ring with the aminothiazole was desirable for maximal

* Corresponding author. Tel.: +1 860 686 2123.
E-mail address: mark.mitton-fry@pfizer.com (M. Mitton-Fry).
† Present address: Alkermes, Inc., 88 Sidney Street, Cambridge, MA 02139, USA.
‡ Current address: Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA.
§ Current address: SNBL USA, Ltd, 6605 Merrill Creek Parkway, Everett, WA 98203, USA.

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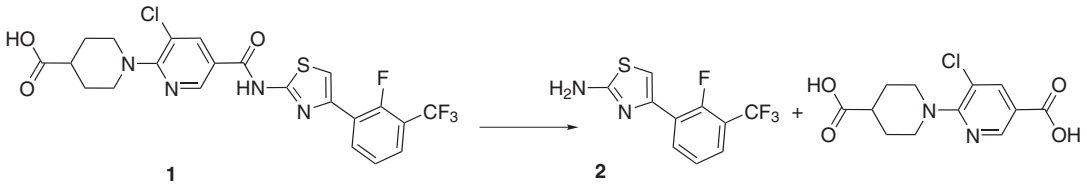


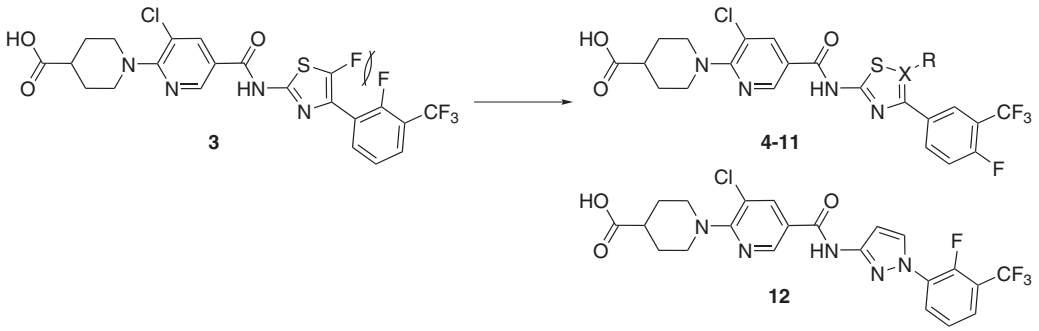
Figure 1.

Table 1
Pharmacokinetic and serum chemistry analysis from single oral doses of compounds **1** and **5**[a]

Compound	Dose (mg/kg)	Mean AST (IU/L)	Mean ALT (IU/L)	Mean C_{max}[b] (μg/mL)	Mean AUC_{0-24}[b] (μg h/mL)
1	0	96	55	0	0
1	50	1937	442	20	84
1	250	2952	450	48	832
1	500	3495	579	84	1486
5	0	102	56	0	0
5	50	87	58	50	548
5	250	897	202	120	1818
5	500	1899	337	280	4736

[a] These were conducted as separate studies, each with their own control group (entries 1 and 5)
[b] All concentrations refer to total concentrations.

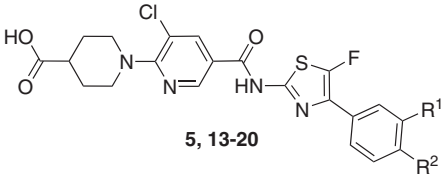
Table 2



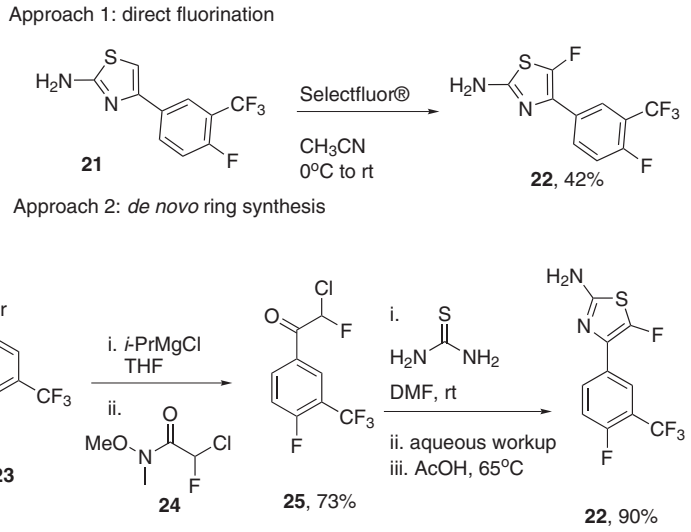
Compound	R	X	BaF_3·EC_{50} (μM)
1			0.033
3			0.702
4	H	C	0.1
5	F	C	0.089
6	CH_3	C	0.066
7	Cl	C	0.086
8	CH_2CH_3	C	0.16
9	$(CH2)_2CH_3$	C	0.13
10	CN	C	>0.898
11		N	0.574
12			>0.811

potency.[10] Thus, adding a substituent at C-5 of the thiazole, as in **3**, likely leads to an unfavorable biaryl ring orientation owing to a steric and/or electronic clash between the C-5 thiazole and the C-2 phenyl fluoro substituents. In order to relieve this undesirable conformation effect, the C-2 phenyl substituent would have to be removed. Previous efforts in a pyrimidine benzamide series of TPO receptor agonists had revealed that the 4-F-3-CF_3-phenyl substitution pattern was only about 2-fold less active than the 2-F-3-CF_3 substitution pattern.[11] Indeed, incorporation of this substitution pattern into the isonipecotic acid series led to a compound (**4**) only about 3-fold less potent than **1**. Doing so in conjunction with a thiazole C-5 fluoro group likewise yielded a potent agonist, **5**. A number of other nonpolar thiazole C-5 substituents led to similarly potent compounds, **6–9**. Of the substituents examined only the polar cyano group led to a compound with significantly reduced potency (**10**). Altering the heterocycle to either a thiadiazole (**11**) or a pyrazole (**12**) also led to significantly lower

Table 3



Compound	R^1	R^2	BaF_3·EC_{50} (μM)
5	CF_3	F	0.089
13	Cl	F	0.48
14	Br	F	0.67
15	Cl	Cl	>1.0
16	OCF_3	F	0.62
17	OCH_2CF_3	F	0.95
18	CH_2OEt	F	>0.97
19	Butyl	F	0.22
20	Isobutyl	F	0.058



Scheme 1.

agonist activity, a finding consistent with work in the pyrimidine benzamide series.[11]

Earlier work had established that the optimal agonist activity was obtained with a lipophilic group at C-3 of the phenyl ring and a 2,3-di-, 3,4-di-, or 2,3,4-tri-substitution pattern.[11] We thus examined a limited range of 3,4-disubstitution variations in order to improve potency further. However, with the exception of the 3-isobutyl-4-F derivative **20**, these analogs were significantly less potent (Table 3). Since **20** introduced additional potential sites for metabolism and was more lipophilic than **5** (*C* Log *P* = 5.61 and 4.54, respectively), we chose to focus our efforts on **5**.

In order to synthesize **5**, we developed two approaches for the efficient preparation of key intermediate **22** (Scheme 1). In the first of these, aminothiazole **21** could be directly fluorinated in modest yield using Selectfluor®.[6] In the second approach, **22** could be synthesized using a de novo synthesis of the heterocyclic ring. In this case the bromoarene **23** is first magnesiated and then reacted with the Weinreb amide **24**.[12] The resulting ketone (**25**) was reacted with thiourea[13] and the crude product heated with glacial acetic acid to afford the desired thiazole product in high yield.[14]

As has already been reported,[6] neither **5** nor **22** undergo metabolic activation in an in vitro system. This differentiation was further manifested in lowered hepatic effects in in vivo studies with **5** (Table 1). In contrast to **1**, no changes in either AST or ALT were observed at an oral dose of 50 mg/kg, despite significantly higher exposures of **5**. At higher doses, the increases in AST and ALT were of diminished magnitude relative to those seen with **1**, again despite higher total exposures of **5** versus **1** at each dose level. In four-day studies, 10, 50 and 150 mg/kg daily oral dosing in rats led to no clinical pathology, and only mild increases in ALT (one animal of four on day 1) and bilirubin (one animal of four on day 5) were observed. No histopathological changes were found at any dose.

In order to put these results into proper context, an evaluation of in vivo efficacy was warranted. Our proprietary series of small molecules, and those of others, are functional agonists of the human TPO receptor. However, they have been found to be inactive when tested against non-human animal species of bone marrow cells, due to a unique histidine found only in the human receptor's transmembrane domain.[15] These findings precluded the usual preclinical pharmacological evaluation of these compounds in standard laboratory animal species. In order to develop an animal model for in vivo pharmacologic testing of compounds, a murine knock-out/human knock-in of exon 8–10 of the TPO receptor, spanning the transmembrane domain, was created to generate a 'humanized' TPOr transgenic mouse.[7] The human TPO receptor transgenic mice exhibited dose-dependent increases in platelets measured on day 6 after two doses of compound on days 0 and 1, whereas wild-type mice did not. The EC_{50} for compound **1** was 27 μg h/mL, and for compound **5**, it was 7 μg h/mL.

These positive results demonstrate that appropriate substitution at C-5 of the thiazole along with concomitant alteration of the substitution on the phenyl ring can provide potent, orally active TPO receptor agonists[7] with reduced potential for hepatotoxicity in rodent toxicology studies.

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Fullerton, CA). Twenty microliters of cells (10,000 cells per well) were added using a Multi-drop (ThermoElectron Corp, Milford, MA) and the plates were incubated for 5 h at 37 °C, 5% CO_2. Controls included wells with cells plus 150 ng/mL hTPO in assay media, cells plus assay media and assay media only. Ten microliters of LiveBLAzer™-FRET B/G (CCF4-AM) was added to each well and the plates were incubated in the dark, at room temperature for 1 h. The plates were read on a LJL Analyst (Molecular Devices Corporation, Sunnyvale, CA) equipped with the 405–20 excitation filter, two emission filters (blue channel 460–40 and green channel 530–10) and a 425 dichroic filter. A stimulation index (SI) was calculated for each compound using the formula [(460/530 ratio drug samples divided by 460/530 no drug or TPO control ratio)] − 1. An EC_{50} was calculated by plotting SI drug ratio drug to SI hTPO control ratio. Full experimental details are described in WO2007036769.

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