Bioorganic & Medicinal Chemistry Letters 19 (2009) 1428–1430

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The identification of orally bioavailable thrombopoietin agonists

Michael J. Munchhof *, Amy S. Antipas, Laura C. Blumberg, William H. Brissette, Matthew F. Brown, Jeffrey M. Casavant, Jonathan L. Doty, James Driscoll, Thomas M. Harris, Lilli A. Wolf-Gouveia, Christopher S. Jones, Qifang Li, Robert G. Linde, Paul D. Lira, Anthony Marfat, Eric McElroy, Mark Mitton-Fry, Sandra P. McCurdy, Lawrence A. Reiter, Sharon L. Ripp, Andrei Shavnya, Lisa M. Thomasco, Kristen A. Trevena

Pfizer Global Research and Development, Groton Laboratories, MS8220-2405, Eastern Point Road, Groton, CT 06340, USA

ARTICLE INFO

Article history:
Received 1 December 2008
Revised 6 January 2009
Accepted 12 January 2009
Available online 15 January 2009

Keywords:
TPO
Thrombopoietin
Agonist

ABSTRACT

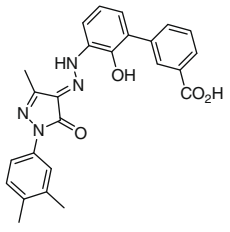
Recently, we disclosed a series of potent pyrimidine benzamide-based thrombopoietin receptor agonists. Unfortunately, the structural features required for the desired activity conferred physicochemical properties that were not favorable for the development of an oral agent. The physical properties of the series were improved by replacing the aminopyrimidinyl group with a piperidine-4-carboxylic acid moiety. The resulting compounds possessed favorable in vivo pharmacokinetic properties, including good bioavailability.

The discovery of agonists of the thrombopoietin receptor (TPOr) is an active area of research within the pharmaceutical industry for the treatment of a number of conditions resulting in suppressed platelet levels.[1] The FDA recently approved Eltrombopag, shown in Figure 1, as the first thrombopoietin agonist for the treatment of idiopathic thrombocytopenic purpura (ITP).[2]

We recently reported a novel series of potent TPOr agonists exemplified by **1**.[3] Unfortunately, the structural features required for the desired activity conferred physicochemical properties that were not favorable for the development of an oral agent.[4] The poor solubility of the series made permeability, as well as other in vitro properties such as metabolic stability, difficult to evaluate.

Earlier analoging efforts suggested that one way to obtain the desired agonist activity was to maintain planarity from the amide to the thiazole and through the pendent 2-fluoro-3-(trifluoromethoxy)phenyl ring of **1**.[3,5] Efforts to modify this region of the pharmacophore while preserving the planarity had previously met with little success.[5] As a result, we turned our attention to replacements for the diaminopyrimidine portion of the lead. A pharmacophore model of TPO agonism was also used, which suggested that a hydrogen bond acceptor was favored in the terminus of the phenylpyrimidine-4,6-diamine, portion of the molecule.[5] We therefore sought to identify structural groups that would remove the planar diaminopyrimidine and replace it with a heterocycloalkyl group that displayed the required hydrogen bond acceptor. Additionally, we replaced the phenyl benzoic acid moiety with the corresponding nicotinic acid to help reduce lipophilicity and facilitate analog preparation. Combined, these changes were pursued in an attempt to move to a more drug-like physical property space, including better solubility and more polar compounds (Fig. 2).

Based on these criteria a variety of replacements were screened, resulting in the identification of the pyrrolidine-3- and piperidine-4-carboxylic acids **2** and **3**.[6,7] Acid **2** had excellent agonist activity in the BaF_3 reporter assay (EC_{50} = 33 nM), but showed only a slight improvement in solubility as compared to **1**.[8] Compound **3** was not as potent as **2**, but displayed improved aqueous solubility relative to **1** (167 vs <1 μg/mL). Unfortunately, improved solubility of **3**, coupled with moderate permeability in the CACO-2 assay, did not translate to improved oral bioavailability upon administration



Eltrombopag

Figure 1. Eltrombopag.

* Corresponding author. Tel.: +1 860 441 6189.
E-mail address: Michael.j.munchhof@pfizer.com (M.J. Munchhof).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.01.032

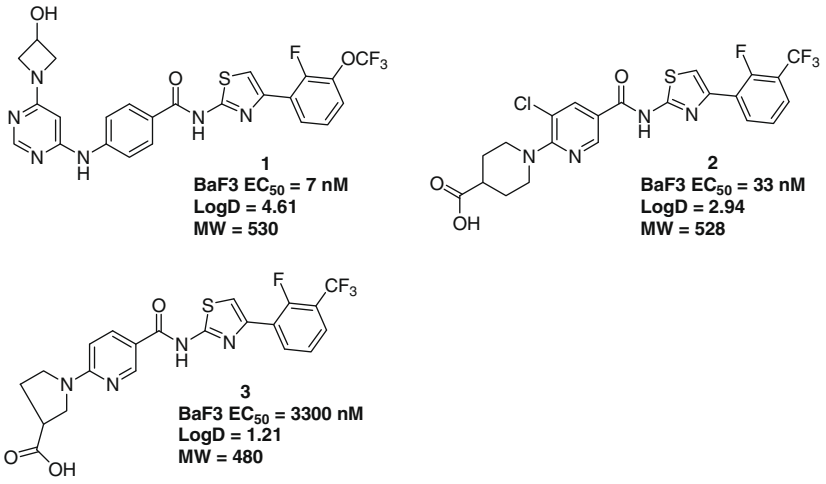


Figure 2. Lead compounds.

in rats ($F < 3\%$). We incorrectly concluded at the time that a further improvement in solubility was required.[9]

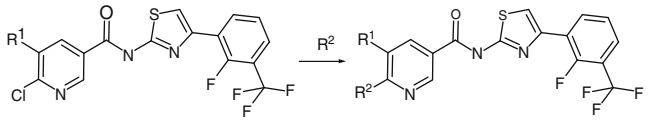
Because of the superior potency of the 3-chloro group and piperidine-4-carboxylic acid combination, we chose to focus further work on this series. Thus, we next investigated modification of the piperidine-4-carboxylic acid moiety. Analogs were prepared by addition of amines to dichloropyridyl amide **4** (Table 1). Replacement of the piperidine-4-carboxylic acid with carboxamides, sulfonamides, sulfonic acids, and carboxylic acid isosteres such as tetrazoles all yielded compounds that maintained a range of agonist activities. Acyclic acids such as **8**, as well as the piperazine-3- and piperidine-3-carboxylic acids **9** and **10**, were also moderately potent. Removing the hydrogen bond acceptor of **2** to afford piperidine **13** resulted in loss of activity ($EC_{50} > 10\ \mu M$). These results are consistent with our hypothesis that a hydrogen bond acceptor is important for activity and demonstrated that some flexibility with regard to its general spatial placement can be tolerated.

We next turned our attention to the pyridine moiety. Replacement of the pyridine ring with less basic pyrimidine and pyrazine moieties led to inactive compounds (data not shown). These results suggested that the basicity of the pyridine nitrogen may be important for activity. To explore this possibility, several 3-substituted pyridine analogs were prepared with both electron donating and withdrawing substituents. However, the rank order of potency of these substituted analogs, $Br > Cl > CF_3 > CN > Me > H > F/MeO$ does not correlate with the calculated basicity of the pyridine nitrogen (Table 2). Further evidence that pyridine basicity does not play a critical role was provided by benzene analogs of **2**. Compound **16**, the direct comparator to **2**, is only slightly less potent. Addition of a second chloro group to the benzene ring (**14**) improved the potency to 10 nM. Although the benzene analogs provided useful insight into the SAR, they were compromised by increased lipophilicity. Unfortunately, none of these changes showed a significant improvement in activity or solubility over **2**.[10]

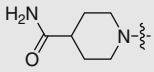
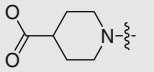
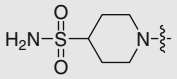
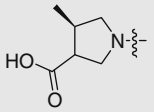
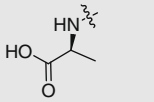
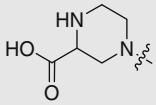
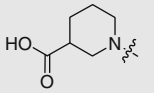
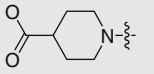
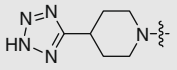
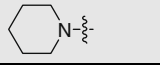
During the course of our studies, Smith Kline Beecham disclosed thrombopoietin agonists of a different chemotype (Fig. 3).[11] Of particular interest was their disclosure of the use of a bis-ethanolamine salt to improve the aqueous solubility of this carboxylic acid-containing compound (**22**, Eltrombopag) from <0.001 to 14 mg/mL.

We prepared the ethanolamine salt of **2** and observed a similar dramatic increase. While the parent acid had no measurable aqueous solubility, the solubility of the ethanolamine salt improved to 15.6 mg/mL. The ethanolamine salt of **2** was pro-

Table 1

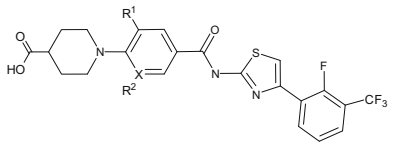


4

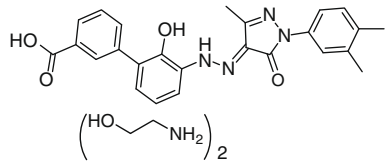
Compound ID	R^1	R^2	BaF_3[a] EC_{50} (μM)	log D	MW
5	Cl		0.028	4.92	527
2	Cl		0.033	2.94	528
6	Cl		0.034	4.86	563
7	Cl		0.043	2.79	528
8	Cl		0.057	1.57	488
9	Cl		0.129	1.88	545
10	Cl		0.294	2.94	528
11	H		0.430	1.83	494
12	Cl		0.485	3.24	552
13	Cl		>10.0	7.1	484

[a] All reported EC_{50} values were calculated by plotting stimulation index ratio of drug, against stimulation index ratio of hTPO control.

Table 2



Compound ID	R^1	X	R^2	BaF_3 EC_{50} (μM)	Calculated Pkb of pyridyl nitrogen
14	Cl	C	Cl	0.01	NA
2	Cl	N		0.033	5.21
15	Br	N		0.050	5.14
16	Cl	C	H	0.056	NA
17	CF_3	N		0.134	5.07
18	CN	N		0.138	4.83
19	Me	N		0.253	7.79
11	H	N		0.430	7.50
20	F	N		>1.00	5.08
21	MeO	N		>1.00	6.94



22

Figure 3. SKB lead.

Table 3
Compound **2** PK data

	Sodium salt	Ethanolamine salt
Rat PK		
Cl_p (mL/min/kg)	6.87	6.34
Vdss (L/kg)	0.831	0.579
$T^{1/2}$ (h)	1.34	1.29
F (%)	66.8	53.4
Dog PK		
Cl_p (mL/min/kg)		0.94
Vdss (L/kg)		0.228
$T^{1/2}$ (h)		2.31
F (%)		50

gressed into rat PK studies in which the bioavailability was found to be 54%, which appeared to substantiate our hypothesis regarding the importance of solubility.[12] Interestingly, however, the sodium salt of **2** was subsequently tested and shown to have comparable oral exposure and clearance properties, even though it had no measurable solubility.[13] This trend held for several related acids but was not true in every case.[14] Clearly, although the replacement of diaminopyrimidine portion of **1** had not led to the desired increase in solubility, it did confer improved properties relative to the lead, that resulted in favorable in vivo pharmacokinetic properties.

Having achieved good oral exposure with **2**, we further assessed the attributes of the compound. PK studies in dogs also showed good bioavailability, low clearance, and low volume of distribution.[15] Incubation in human liver microsomes resulted in no metabolic turnover, and no reactive metabolites were detected. As expected for a lipophilic carboxylic acid, **2** was highly bound (>99.5%) to rat, dog, mouse, and human plasma protein (Table 3).

In summary, the replacement of the diaminopyrimidine portion of **1** with a heterocycloalkyl moiety led to the identification of compounds with much improved in vivo pharmacokinetic profiles. In turn, these advances allowed the program to evaluate the in vivo efficacy of this series in transgenic mouse models.[16]

References and notes

1. For recent related publications and background information on thrombopoietin and thrombopoietin agonists see (a) Revill, P.; Serradell, N.; Bolos, J. *Drugs Future* **2006**, *31*, 767; (b) Alper, Phil B.; Marsilje , Thomas H.; Mutnick, Daniel; Lu, Wenshuo; Chatterjee, Arnab; Roberts, Michael J.; He, Yun; Karanewsky, Donald S.; Chow, Donald; Lao, Jianmin; Gerken, Andrea; Tuntland, Tove; Liu, Bo; Chang, Jonathan; Gordon, Perry; MartinSeidel, H.; Tian, Shin-Shay *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5255; (c) Marsilje , Thomas H.; Alper, Phil B.; Lu, Wenshuo; Mutnick, Daniel; Michellys, Pierre-Yves; He, Yun; Karanewsky, Donald S.; Chow, Donald; Gerken, Andrea; Lao, Jianmin; Kim, Min-Ju; Martin Seidel, H.; Tian, Shin-Shay *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5259.
2. For a recent review of TPO agonists in clinical development for ITP see Stasi, Roberto; Evangelista, Maria L.; Amadori, Sergio *Drugs* **2008**, *68*, 901.
3. Reiter, Lawrence A.; Subramanyam, Chakrapani; Mangual, Emilio J.; Jones , Christopher S.; Smeets, Marc I.; Brissette, William H.; McCurdy, Sandra P.; Lira, Paul D.; Linde, Robert G.; Li, Qifang; Zhang, Fangning; Antipas, Amy S.; Blumberg, Laura C.; Doty, Jonathan L.; Driscoll, James P.; Munchhof, Michael J.; Ripp, Sharon L.; Shavnya, Andrei; Shepard, Richard M.; Sperger, Diana; Thomasco, Lisa M.; Trevena, Kristen A.; Wolf-Gouveia, Lilli A.; Zhang, Liling *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5447.
4. For a review on physiochemical properties and their relationship to oral exposure see Wenlock Mark, C.; Austin, Rupert P.; Patrick, Barton; Davis, Andrew M.; Leeson, Paul D. *J. Med. Chem.* **2003**, *46*, 1250.
5. For a detailed study of the agonism-inducing conformation of this series see Reiter, Lawrence A.; Jones , Christopher S.; Brissette, William H.; McCurdy, Sandra P.; Abramov, Yuriy A.; Bordner, Jon; DiCapua, Frank; Munchhof, Michael J.; Rescek, Diane M.; Samardjiev, Ivan J.; Withka, Jane M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3000.
6. Full experimental details are described in WO2007036769.
7. Kalgutkar, Amit S.; Driscoll, James; Zhao, Sabrina X.; Walker, Gregory S.; Shepard, Richard M.; Soglia, John R.; Atherton, James; Yu, Linning; Mutlib, Abdul E.; Munchhof, Michael J.; Reiter, Lawrence A.; Jones, Christopher S.; Doty, Johnathan L.; Trevena, Kristen A.; Shaffer, Christopher L.; Ripp, Sharon L. *Chem. Res. Toxicol.* **2007**, *20*, 1954.
8. The BaF_3 reporter assay conditions are described in WO2007036769.
9. This conclusion was based on the IV pharmacokinetic data for **3**, that showed the compound had low in vivo clearance (2.5 mL/min/kg), in addition to the fact the compound showed moderate permeability in the CACO-2 (4.5×10^{-6} cm/s) assay.
10. All of the compounds in Tables 1 and 2 had aqueous solubilities of <10 μg/mL.
11. See WO2003098992 for full details.
12. Compound **2** was dosed orally in rats at 5 mg/kg in 0.5% methyl cellulose (aq) (0.5 mg/mL, 10 mL/kg).
13. Earlier in the program we had prepared sodium salts of several acids and seen no improvement in solubility.
14. For example, the ethanol amine salt of **3** had greater than 2 mg/mL solubility, but no detectable bioavailability. The SAR suggested that the piperidine acids may be substrates for active transporters, but we have no experimental evidence to support this.
15. Compound **2** was dosed orally in dogs at 5 mg/kg in 0.5% methyl cellulose (aq) (0.5 mg/mL, 10 mL/kg).
16. Publication in preparation describing the in vivo efficacy of this series. William H. Brissette, Paul D. Lira, Sandra P. McCurdy, Robin T. Nelson, Kuldeep Neote, Jeffrey L. Stock, James P. Driscoll, Kristen A. Trevena, Richard M. Shepard, Christopher S. Jones, Michael J. Munchhof, Lawrence A. Reiter, Sharon L. Ripp.