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New benzimidazoles as thrombopoietin receptor agonists

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Abstract—A novel benzimidazole series of small-molecule thrombopoietin receptor agonists has been discovered. Herein, we discuss the preliminary exploration of structure–activity relationships within this chemotype.

Thrombopoietin (TPO) is a member of an extensive family of extracellular signaling proteins, collectively termed cytokines.[1] TPO plays a critical role in the production of platelets by stimulating the proliferation of megakaryocyte progenitor cells into fully mature megakaryocytes, which then undergo fragmentation to produce platelet bodies.[2] Platelets are essential in the process of blood clotting and repair of damaged blood vessels. Thrombocytopenia, or low circulating platelet levels, can be a debilitating condition associated with a range of medical disorders, and is also a common side effect in cancer patients receiving intensive chemotherapy treatment. Current methods to manage thrombocytopenia suffer from drawbacks such as limited efficacy, serious side effects, and inconvenient routes of administration.[3] Therefore, an orally bio-available non-peptide TPO receptor agonist may be beneficial to patients suffering from thrombocytopenia.

The identification of small molecule mimics of hematopoietic growth factors is an active area of research[4] and several non-peptidyl agonists have been reported.[5] Recently, a rationally designed series of naphtho[1,2-*d*]imidazole TPO receptor agonists, exemplified by structure A (Fig. 1), have been described.[5c] There now exists a large body of evidence for a shared pharmacophore hypothesis between this series and the previously identified classes of TPO mimics.[5c] Figure 1 illustrates the proposed key pharmacophore elements required for potent TPO agonist activity: (1) a lipophilic domain and (2) an acidic moiety on the opposite end of the molecule separated by (3) a heteroatom metal chelate in the central portion.

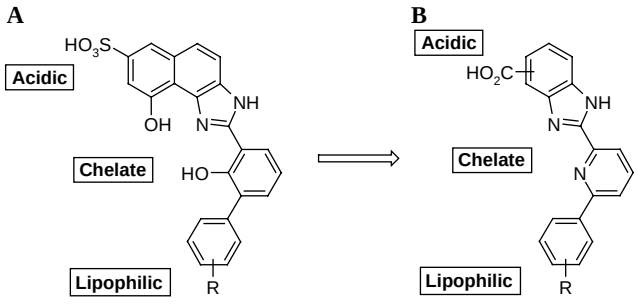

Figure 1. The design of naphtho[1,2-*d*]imidazole (A) and benzimidazole (B) templates.

In our ongoing effort to identify additional small-molecule TPO mimics based on the existing pharmacophore model, we have initiated a study directed toward further optimization of the naphtho[1,2-*d*]imidazole class, with the major emphasis on improving potency and efficacy. We immediately realized that deletion of the terminal aromatic ring represents an obvious and logical simplification of the existing scaffold. As can be seen in Figure 1, the resultant benzimidazole template (B) still incorporates the three pharmacophore requirements (acidic–chelate–lipophilic), although the introduced structural

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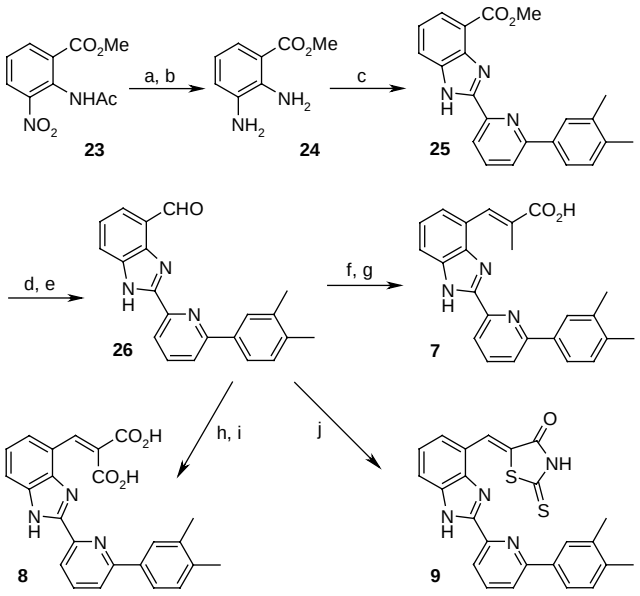
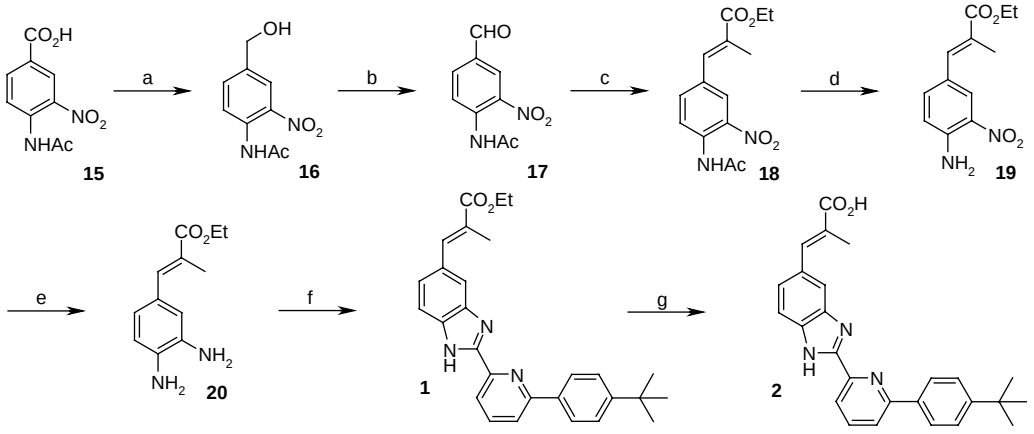
changes inevitably necessitate migration of the acidic moiety closer to the chelate domain and reduce the maximum number of chelatable heteroatoms from three to two. Furthermore, the new framework benefits from elimination of a potential metabolic liability of the phenol and allows for ultimate refinements of developability characteristics without a significant increase in molecular weight.

The 5-substituted benzimidazoles were prepared as described in Scheme 1. Reduction of 4-acetamido-3-nitrobenzoic acid (**15**) with borane–THF complex followed by oxidation with Dess–Martin periodinane[6] of the resultant benzyl alcohol gave aldehyde **17**. Horner–Wadsworth–Emmons reaction of the latter, subsequent base-mediated hydrolysis of the *N*-acetyl group, and reduction of the nitro moiety under mild conditions afforded di-aniline **20** without concomitant hydrogenation of the trisubstituted olefin (43% from **15**). Oxidative cyclocondensation of **20** with sodium hydrogen sulfite ($NaHSO_3$) and 6-(4-*tert*-butyl-phenyl)-pyridine-2-carbaldehyde[7] furnished benzimidazole **1**. Finally, saponification of the ethyl ester provided acid **2**. Benzimidazole **3** was prepared from aldehyde **17** via an analogous sequence of transformations (Scheme 2).

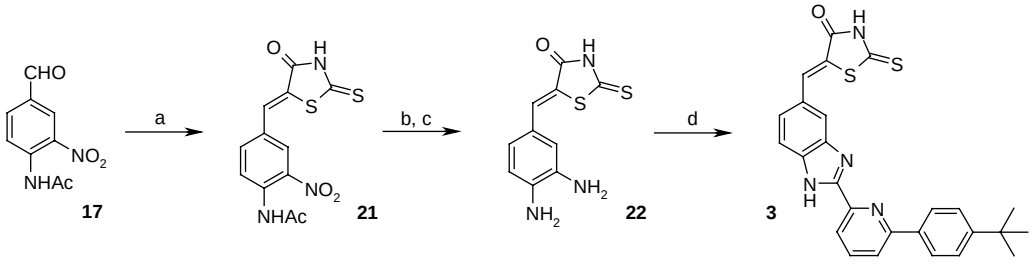
Synthesis of the 4-substituted benzimidazoles commenced from commercially available methyl-2-acetamido-3-nitrobenzoate (Scheme 3). Saponification of the



Scheme 3. Preparation of **7**, **8**, and **9**. Reagents and conditions: (a) K_2CO_3, MeOH, 60 °C, 1 h , 80%; (b) 10% Pd/C, EtOH, 40 °C, 2 h, 95%; (c) 6-(3,4-dimethyl-phenyl)-pyridine-2-carbaldehyde, EtOH, H_2O, $NaHSO_3$, 60 °C, 64%; (d) $LiAlH_4$, THF, $-78 \rightarrow 0$ °C, 98%; (e) Dess–Martin periodinane, CH_2Cl_2, pyridine, 83%; (f) (carbethoxyethyl)triphenylphosphorane, CH_2Cl_2, rt, 95%; (g) MeOH, NaOH, 50 °C, 15 h, 71%; (h) diethyl malonate, PhH, piperidine, reflux, 15 h, 100%; (i) THF, NaOH, 50 °C, 15 h, 64%; (j) rhodanine, EtOH, piperidine, reflux, 15 h, 84%.



Scheme 1. Preparation of **1** and **2**. Reagents and conditions: (a) BH_3·THF, THF, 0 °C to rt, 57%; (b) Dess–Martin periodinane, CH_2Cl_2, pyridine, 83%; (c) (carbethoxyethylidene)triphenylphosphorane, CH_2Cl_2, rt, 95%; (d) K_2CO_3, EtOH, reflux, 1 h, 96%; (e) PtO_2, EtOAc, H_2 (1 atm), rt, 100%; (f) 6-(4-*tert*-butyl-phenyl)-pyridine-2-carbaldehyde, EtOH, H_2O, $NaHSO_3$, 60 °C, 73%; (g) MeOH, NaOH, 50 °C, 89%.



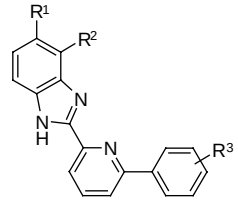
Scheme 2. Preparation of **3**. Reagents and conditions: (a) rhodanine, EtOH, piperidine, reflux, 15 h, 94%; (b) K_2CO_3, EtOH, reflux, 1 h, 96%; (c) $SnCl_2$, concd HCl, 100 °C, 2.5 h, 96%; (d) 6-(4-*tert*-butyl-phenyl)-pyridine-2-carbaldehyde, EtOH, H_2O, $NaHSO_3$, 60 °C, 47%.

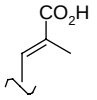
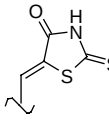
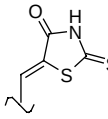
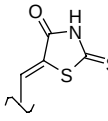
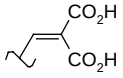
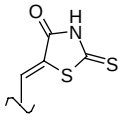
acetyl group, followed by catalytic hydrogenation of the nitro moiety and oxidative cyclocondensation with 6-(3,4-dimethyl-phenyl)-pyridine-2-carbaldehyde-provided benzimidazole **25** (49%, 3 steps). Reduction of the ester moiety with LAH and Dess–Martin oxidation afforded aldehyde **26**, which was subjected to a Knoevenagel reaction with diethylmalonate and rhodanine to give **8** and **9**, respectively. A Horner–Wadsworth–Emmons reaction of **26** with (carboethoxyethylidene)triphenylphosphorane gave **7** after saponification of the ester.

The TPO receptor agonist activity of the benzimidazoles was tested in vitro by their ability to induce proliferation of the murine Ba/F3-hTpoR hematopoietic cell line. These cells express the human TPO receptor, which confers a proliferative response to TPO stimulation.[8]

We first investigated the effect of variations in the acidic domain of the pharmacophore, in terms of the nature of functionality and substitution pattern on the benzimidazole nucleus (Table 1). In accord with the pharmacophore model, 5-substituted acrylic ester **1** totally lacked proliferative activity while acid **2** was active, albeit modestly. Interestingly, the malonic acid derivative **3** was less active, suggesting that either the conformational restriction provided by the double bond in **2** was a feature required for activity or that the presence of the second carboxylic acid group was a detrimental factor. Incorporation of the thioxo-thiozolidinone (rhodanine) group, which can be considered as a carboxylic acid isostere,[9] improved the potency (compounds **4**–**6**). There also appeared to exist a preference, with respect to the level of proliferation relative to maximum TPO, for

Table 1. Effect of variation in the acidic domain of benzimidazoles on TPO mimetic activity



Compound	R^1	R^2	R^3	EC_{50} (μM)	Efficacy[a] (% TPO)
1		H	4-$C(CH_3)_3$	NA[b]	—
2		H	4-$C(CH_3)_3$	4.5	109
3		H	4-$C(CH_3)_3$	10	13
4		H	4-$C(CH_3)_3$	2	90
5		H	3,4-CH_3	1.6	73
6		H	3,4-Cl	1.0	19
7	H		3,4-CH_3	NA	—
8	H		3,4-CH_3	NA	—
9	H		3,4-CH_3	NA	—

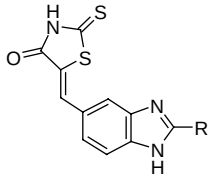
[a] Efficacy is defined as a percentage of the proliferation value induced by maximal TPO concentration [(TPO) ≈ 0.1 ng/mL].
[b] NA, not active.

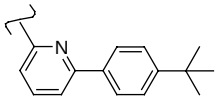
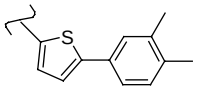
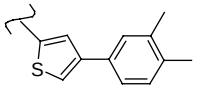
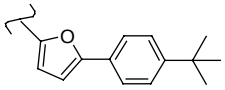
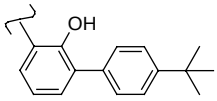
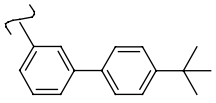
bulkier lipophilic R^3 substituents on the terminal aromatic ring in these analogues. As far as the 4-substituted benzimidazoles are concerned, all lacked activity regardless of the chemical nature of the acidic domain (compounds **7**–**9**). This observation points to a plausible conclusion that excessive clustering of the pharmacophore elements renders such structures inactive.

Next, we proceeded to determine SAR trends in the hypothesized metal-binding domain of the pharmacophore. As can be seen in Table 2, a variety of heteroatom-containing biaryl substituents (compounds **10**–**13**), which are weak metal chelators, were equally well tolerated, furan **12** being the most potent analogue. Surprisingly, compound **14**, devoid of any heteroatoms incorporated in the biaryl group (R), had similar potency and level of effect to the other benzimidazole TPO receptor agonists described, indicating that the N-1 nitrogen of the benzimidazole ring alone can function as the putative metal-binding domain for these TPO receptor agonists.

We have discovered a new benzimidazole series of small-molecule TPO mimetic agents. A preliminary screen of functional group tolerances around the benzimidazole core revealed a tight SAR pattern with only modest TPO agonist activity, as compared to the parent naphtho[1,2-*d*]imidazole class.[5c] In light of the unexpected activity of compound **14**, our current understanding of the pharmacophore model, as applied to benzimidazole TPO agonists, requires a re-evaluation. The full potential of this chemotype with respect to thrombopoietic activity as well as further refinements of the developability profile are yet to be explored and will be reported in due course.

Table 2. Effect of variation in the metal chelate domain of benzimidazoles on TPO mimetic activity



Compound	Bi-aryl group (R)	EC_{50} (μM)	Efficacy[a] (% TPO)
4		2	90
10		1.75	117
11		0.78	123
12		0.72	142
13		1.0	134
14		0.9	137

[a] Efficacy is defined as a percentage of the proliferation value induced by maximal TPO concentration [(TPO) ≈ 0.1 ng/mL].

References and notes

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7. All bi-aryl aldehydes used to prepare benzimidazoles **1**–**14** are either known compounds themselves or can be synthesized in one step from known/commercially available components by way of a general Suzuki coupling protocol.
8. Ba/F3-hTpoR cells, a murine B lymphocyte cell line transfected with the human TPO receptor, were maintained in RPMI-1640/10% FBS with 5 ng/mL rmIL-3 (R&D Systems) and 500 μg/mL G418 (Gibco) at 37 °C (5% CO_2, 95% relative humidity). Cells were washed and plated at 2×10^5 cells/mL with 0–30 μM compound or rhTPO (0.1% DMSO final concentration) in media. Proliferation was measured by addition of 10 μCi/mL of [methyl-^{3}H]thymidine (Amersham Biosciences) after 72 h in culture. Cells were lysed with water after a further 4 h and harvested using a Brandel 96 Harvester onto a glass fiber filter (Wallac), according to the manufacturer's protocol. MeltiLex A

scintillator sheets (Wallac) were melted onto the dried filters using a TTray Heatsealer (Wallac) and counted in a Wallac 1205 Betaplate reader. The mean proliferation value for quadruplicate wells was plotted versus compound concentration, and EC_{50} values were then calculated. The greatest mean proliferation value for each compound was expressed as a percentage of that induced by maximal TPO concentration in order to calculate effect relative to TPO_{max}.

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