Identification of a Pharmacophore for Thrombopoietic Activity of Small, Non-Peptidyl Molecules. 2. Rational Design of Naphtho[1,2-*d*]imidazole Thrombopoietin Mimics

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Received May 6, 2002

Abstract: The invention of a new class of naphtho[1,2-*d*]-imidazole thrombopoietin mimics based on a pharmacophore hypothesis for small-molecule thrombopoietic agonists is discussed. Parallel array synthesis and purification techniques allowed for the rapid exploration of structure−activity relationships within this class and for the improvement in TPO mimetic potencies and efficacies.

In the preceding paper,[1] we disclosed the discovery of a novel series of salicylaldehyde thiosemicarbazone thrombopoietin (TPO) mimics and the optimization of their potency based on structural comparisons with the known, potent pyrazol-4-ylidenehydrazine class of TPO mimics.[2] The successful use of structure−activity relationships from the pyrazol-4-ylidenehydrazines to improve potency in the salicylaldehyde thiosemicarbazones is very strong evidence that these two structurally diverse series of TPO agonists share similar or identical pharmacophores. Figure 1 summarizes the three key structural features previously identified for potent TPO agonist activity: (1) lipophilic functionality and (2) acidic functionality on opposite ends of the molecule separated by (3) a heteroatom metal chelate in the central portion. Encouraged by this previous successful implementation of our pharmacophore hypothesis for small-molecule TPO mimics, we initiated studies toward the rational design of a further structurally distinct series of TPO mimics using these three structural elements (acidic−chelate−lipophilic).

Of the many possible structures considered, the synthetic accessibility of a naphtho[1,2-*d*]imidazole template prompted us to investigate the viability of 2-substituted 9-hydroxynaphtho[1,2-*d*]imidazoles as potential new templates. Figure 2 shows the important structural features of this new template in relation to our pharmacophore hypothesis. The chelation motif is provided by the imidazole N-1, the 9-hydroxy group, and an *o*-hydroxyaryl substituent at C-2. Acidic and lipophilic groups can be readily incorporated at distal ends of the template on the naphthalene ring and on the 2-hydroxyaryl group, respectively. From a synthetic point of view acidic-substituted naphthalenes are more readily available than lipophilic naphthalenes, and so commercially available 4-amino-5-hydroxynaphthalene-1-sulfonic acid **3a** and 5-amino-4-hydroxynaphthalene-2-sulfonic acid **3b** were chosen as initial starting materials (Scheme 1). To prepare the required intermediate *o*-diaminonaphthols **4a**,**b**, naphthalenes **3a**,**b** were coupled with diazotized 4-aminobenzenesulfonic acid to afford the intermediate diazo compounds, which were then reduced with sodium dithionite to afford the desired diamines **4a**,**b** isolated as the dihydrochloride salts. 2-Hydroxy-1-naphthaldehyde and 1-hydroxy-2-naphthaldehyde were selected as representative lipophilic hydroxyarylaldehydes, and their condensations with diamines **4a**,**b** were investigated. Oxidative cyclocondensation of the two isomeric aldehydes with diamines **4a**,**b** with DDQ or sodium hydrogen sulfite then furnished the desired naphtho[1,2-*d*]imidazoles **5a**,**b** and **6a**,**b**.

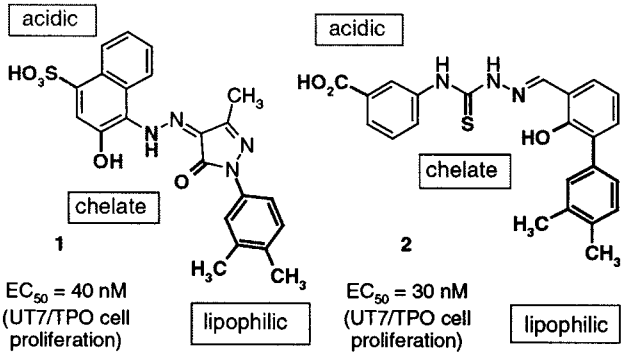

Figure 1. Comparison of pyrazol-4-ylidenehydrazine TPO mimic **1** and thiosemicarbazone TPO mimic **2**, illustrating the three key features for potent thrombopoietic activity: central metal chelate, lipophilic end portion, and a distal acidic functionality.

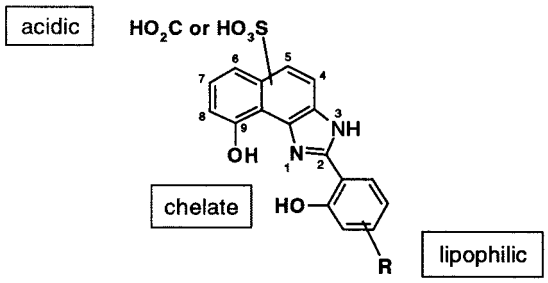

Figure 2. Design of the naphtho[1,2-*d*]imidazole template.

From tests in the BAF-3/TPO-Rluc cell line,[3−5] luciferase assay indeed showed that naphtho[1,2-*d*]imidazoles **5a** and **6a** were TPO mimics with potencies of 35 and 4.8 μM, respectively and efficacies in the range of 30% TPO_{max}. The potencies for the 7-sulfonic acids **5b** and **6b** were 26 and 9.7 μM, respectively, and are of similar magnitude compared to the 5-sulfonic acid isomers. Although the activities for these two compounds were relatively weak when compared to the other two structural classes of TPO mimics, they did

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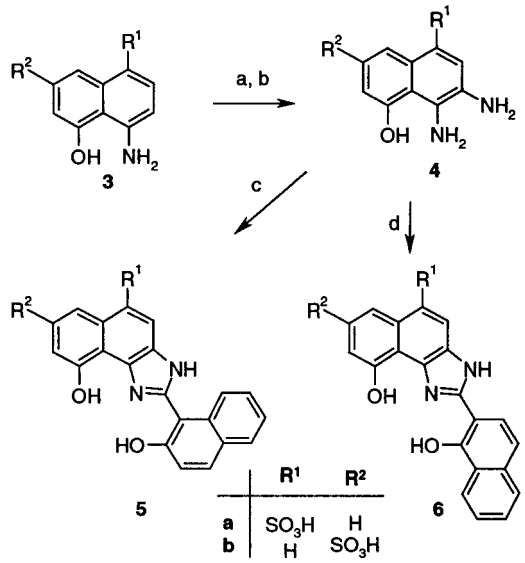
10.1021/jm0255365 CCC: $22.00
Published on Web 07/19/2002

Scheme 1. Synthesis of Naphtho[1,2-*d*]imidazoles **5** and **6**[a]



[a] (a) $NH_2-(p\text{-}C_6H_4)SO_3H$, $NaNO_2$, aqueous HCl, room temp; (b) $Na_2S_2O_7$, aqueous HCl, 50 °C; (c) HO−(naphthyl)−CHO, DDQ, pyridine, MeOH, reflux; (d) HO−(naphthyl)−CHO, $NaHSO_3$, EtOH, water, reflux.

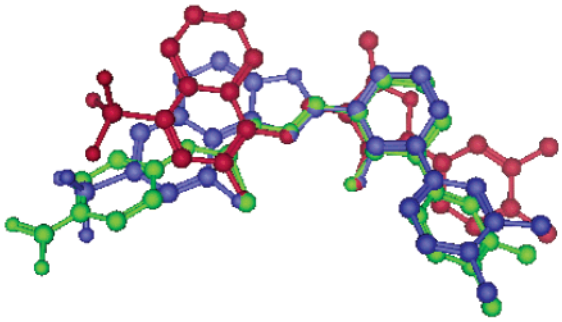


Figure 3. Overlay of the metal-chelating heteroatoms of pyrazol-4-ylidenehydrazine **1** (red) thiosemicarbazone **2** (green) and the naphtho[1,2-*d*]imidazole **8** (blue).[5]

serve to validate our current hypothesis regarding a shared pharmacophore for small-molecule mimetics of TPO.

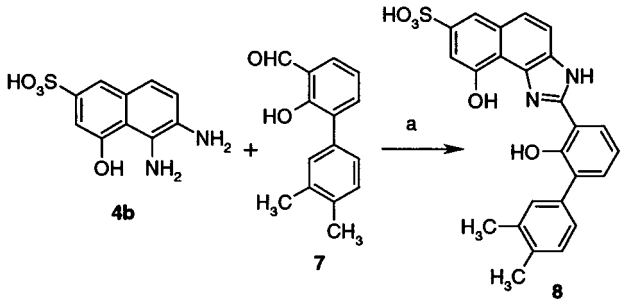
In a strategy similar to that undertaken with the thiosemicarbazone TPO mimics,[1] biphenyl groups were chosen as isosteric replacements of the *N*-arylpyrazolinone functionality of the potent pyrazol-4-ylidenehydrazine class of TPO mimics and were anticipated to be an improvement over the naphthol functionality already incorporated in compounds **5** and **6**. Modeling predicts that lipophilic 3-biaryl functionality would be optimum (Figure 3) as illustrated by the overlay of pyrazol-4-ylidenehydrazine **1**, salicylaldehyde thiosemicarbazone **2**, and a naphtho[1,2-*d*]imidazole substituted at the 2-position with a 3-(3,4-dimethylphenyl)-2-hydroxyphenyl group, compound **8**.

Indeed, biphenyl **8** proved to be more potent and efficacious than the naphthol analogues **5** and **6**. Table 1 compares the potencies and efficacies of naphtho[1,2-*d*]imidazoles **5a**,**b**, **6a**,**b**, and **8** in the BAF-3/TPO-Rluc cell line and toward the proliferation of UT7/TPO cell lines.[7] As can be seen in Table 1, naphthols **5a**,**b** and **6a**,**b** all showed activation of luciferase expression in the BAF-3/TPO-Rluc cell line but lacked sufficient

Table 1. TPO Mimetic Activity for Naphtho[1,2-*d*]imidazoles **5a**,**b**, **6a**,**b**, and **8** As Shown by the Activation of Luciferase Expression in the BAF-3/TPO-Rluc Cell Line and in the Proliferation of the UT7/TPO-R Cell Line

	luciferase		proliferation	
compound	efficacy (% TPO)	EC_{50} (μM)	efficacy (% TPO)	EC_{50} (μM)
5a	52	35	<10	
5b	11	26	<10	
6a	28	4.8	<10	
6b	27	9.7	<10	
8	36	1.2	70	1

Scheme 2. Synthesis of Naphtho[1,2-*d*]imidazole **8**[a]



[a] (a) $NaHSO_3$, EtOH, water, reflux.

agonist activity to effectively support the proliferation of UT7/TPO cell lines during the longer 3-day proliferation assay. However, biphenyl analogue **8** was 5- to 30-fold more potent in the luciferase assay than the naphthols and consequently showed good activity in the UT7/TPO proliferation assay with an EC_{50} of approximately 1 μM.

To expand upon this potency increase, several multicomponent arrays comprising over 300 compounds overall were rapidly synthesized containing different functionality in the biphenyl portion of the naphtho[1,2-*d*]imidazoles and differing in the nature (sulfonic, carboxylic, tetrazole, acyl sulfonamide, etc.) and position (5- and 7-substituted) of the acidic functionality (a full account is outside the scope of this letter and will be published shortly[6]). Illustrative is the synthesis of one member, the 2-[3-(3,4-dimethylphenyl)-2-hydroxyphenyl]naphtho[1,2-*d*]imidazole **8** shown in Scheme 2. The requisite aldehyde **7** was prepared as described previously[1] and oxidatively condensed with the diamine **4b** to provide the biphenylnaphtho[1,2-*d*]imidazole **8** in high yield, isolated as the hydrochloride salt (Scheme 2).

Figure 4 illustrates the SAR trends gathered from the over 300 naphtho[1,2-*d*]imidazoles wherein one intriguing divergence from those of the other two series is conspicuous: the 2-methoxy analogues (Figure 4; X = OMe) were generally more potent than the 2-hydroxy analogues (Figure 4; X = OH), the opposite trend as seen for the thiosemicarbazones.[1] This prompted the synthesis of des-hydroxy analogues (Figure 4; X = H) to investigate whether the third chelatable heteroatom could be completely deleted in the naphtho[1,2-*d*]imidazole series. The 3-(4-*tert*-butylphenyl) group was chosen as one of the optimal lipophilic substituents, and both 7-sulfonic and 7-carboxylic acid[8] analogues were prepared.

As can be seen in Table 2, deletion of the lower heteroatom does result in significant increases in po-

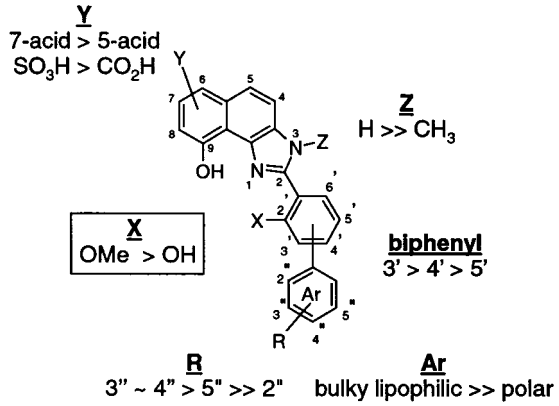
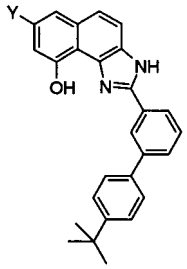


Figure 4. General SAR trends elucidated from array syntheses of over 300 naphtho[1,2-*d*]imidazoles.

Table 2. TPO Mimetic Activity for Naphtho[1,2-*d*]imidazoles **9** and **10** As Shown by the Proliferation of the UT7/TPO-R Cell Line



compound	Y	efficacy (% TPO)	EC_{50} (μM)
9	SO_3H	100	0.08
10	CO_2H	60	0.30

tency in both the sulfonic acid **9** and carboxylic acid **10** naphtho[1,2-*d*]imidazoles over the hydroxy-containing compounds (e.g., compound **8**). This leads to an intriguing conclusion that the pharmacophore requirements are quite similar but not identical for the naphtho[1,2-*d*]imidazole series of TPO mimics compared with the pyrazol-4-ylidenehydrazine and thiosemicarbazone series.

As with these two previously described series of TPO mimics, the naphtho[1,2-*d*]imidazoles are completely selective for TPO-R over other cytokine responsive cell lines (e.g., EPO, G-CSF, IL-3). They also stimulated a similar pattern of cell-signaling protein phosphorylation (STAT-5, MAPK) as TPO itself, and the more potent analogues are able to promote the growth and differentiation of purified $CD34^+$ cells from human bone marrow into CD41 expressing megakaryocytic precursor cells as shown for carboxylic acid analogue **10** in Figure 5.

In this manuscript, we have provided strong evidence that a defined pharmacophore exists for activation of the TPO receptor by small-molecule agents. A full account of the application and further refinement of this pharmacophore model toward the optimization of potency and efficacy of the naphtho[1,2-*d*]imidazole series of TPO mimics introduced here will be reported shortly. Further, a full investigation into the metal binding aspects of the pharmacophore and into the mode of interaction of the TPO mimics with the TPO receptor is beyond the scope of this manuscript.[9]

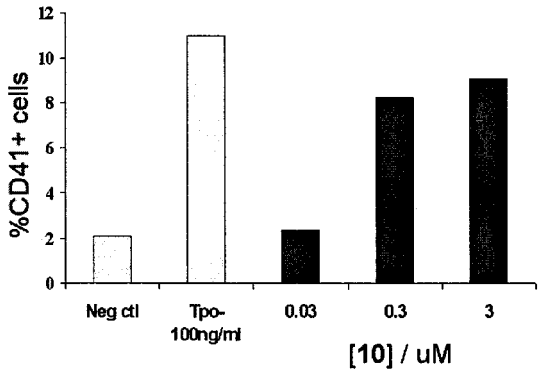


Figure 5. $CD41^+$ assay for **10**. Human bone marrow $CD34^+$ cells (2×10^6 cells/mL) in IMDM containing fetal calf serum (20% v/v) and recombinant human stem cell factor (100 ng/mL) were incubated with **10** for 10 days and then stained with FITC-anti-IgG1 isotype control and FITC-anti-CD41 (Pharmingen clone SZ22) and analyzed on a Becton-Dickinson FACScan flow cytometer.

Supporting Information Available: Experimental details for the synthesis and characterization of naphtho[1,2-*d*]-imidazole TPO mimics **5a**,**b**, **6a**,**b**, and **8**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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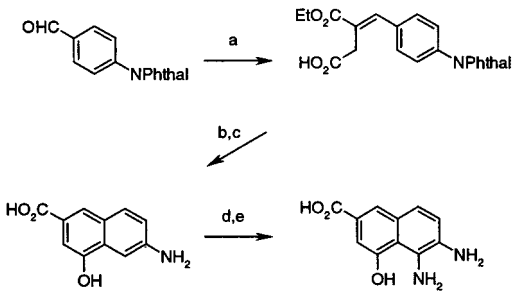
(8) The requisite 5,6-diamino-4-hydroxynaphthalenecarboxylic acid was prepared as shown below. Full experimental procedures are given in the Supporting Information.

(9) Duffy, K. J.; et al. Manuscript in preparation.



(a) $(EtO_2CCH_2)_2$, KOt-Bu, t-BuOH, reflux, 79% (b) NaOAc, Ac_2O, reflux then hydrazine, room temp. 69%; (c) 6N aqu. HCl, reflux 54%; (d) $NH_2SO_2(p\text{-}C_6H_4)\text{-}N_2^+Cl^-$, aqu. HCl, room temp.; (e) $SnCl_2$, conc. HCl, 95 °C, 43% over 2 steps.

JM0255365