Hydrazinonaphthalene and Azonaphthalene Thrombopoietin Mimics Are Nonpeptidyl Promoters of Megakaryocytopoiesis

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High-throughput screening for the induction of a luciferase reporter gene in a thrombopoietin (TPO)-responsive cell line resulted in the identification of 4-diazo-3-hydroxy-1-naphthalenesulfonic acids as TPO mimics. Modification of the core structure and adjustment of unwanted functionality resulted in the development of (5-oxo-1,5-dihydropyrazol-4-ylidene)hydrazines which exhibited efficacies equivalent to those of TPO in several cell-based assays designed to measure thrombopoietic activity. Furthermore, these compounds elicited biochemical responses in TPO-receptor-expressing cells similar to those in TPO itself, including kinase activation and protein phosphorylation. Potencies for the best compounds were high for such low molecular weight compounds (MW < 500) with EC_{50} values in the region of 1−20 nM.

Introduction

Thrombopoietin. The circulatory cytokine thrombopoietin (TPO) plays a critical role in the regulation of megakaryocytopoiesis and is the primary regulator of platelet production.[1] Although the existence of TPO was demonstrated 40 years ago,[2] purification of the protein was achieved only relatively recently.[3−5] Human TPO is a 332 amino acid protein with a 154 amino acid N-terminal region arranged in the classic cytokine motif of four antiparallel α-helices. This N-terminal region has 23% sequence identity with the red blood cell promoter erythropoietin (EPO), but unlike EPO, TPO contains a C-terminal region with no sequence similarity to other known proteins and which is not required for full biological activity. It is believed that this C-terminal region is necessary to stabilize TPO in vivo against proteolytic breakdown and/or facilitate the secretion of TPO.[6] TPO acts through binding and activating the cell-surface receptor c-mpl (the TPO receptor), which is expressed on hematopoietic stem cells, immature hematopoietic progenitor cells, and megakaryocytes. Binding of TPO to c-mpl initiates a cell-signaling cascade involving several pathways, including the activation of JAK-2,[7] STAT-5,[8] and MAPK,[9] leading to the irrevocable commitment of the progenitor cell along the megakaryocytic lineage. Mature megakaryocytes then undergo cell fragmentation to produce the platelet bodies which are an essential component in the process of wound healing through their subsequent cross-linking with fibrin, and the eventual formation of a protective thrombus in compromised blood vessels. The TPO receptor is also expressed on platelets, therefore providing a biological feedback mechanism to inversely regulate the concentration of free, circulating TPO with respect to platelet count.

Thrombocytopenia. Thrombocytopenia is a serious complication in patients receiving intensive chemotherapy. Likewise, a number of other factors can also contribute to thrombocytopenia, conditions such as platelet immunogenicity (ITP),[10] liver dysfunctions, drug treatments (IFN,[11] heparin[12]), and viral infections (HIV,[13] hepatitis C[14]). Currently, management of thrombocytopenia is primarily based on platelet transfusion, which is becoming increasingly costly.[15−17] Patients receiving intensive chemotherapy or myeloablative regimens as treatments for a variety of cancers require repeated transfusions of platelets accounting for approximately 40% of the platelet transfusions performed in the U.S. at this time.[18−20] Although variable, the standard level to date before administration of a transfusion is approximately 5000−20000 platelets/uL,[21,22] and at such low levels bleeding time can be prolonged almost indefinitely. Despite their effectiveness, platelet transfusions can lead to numerous, serious complications including alloimmunization (therefore rendering the patient refractory to subsequent transfusions), febrile and allergic responses, circulatory overload, and bacterial or viral infections (rare today but fatal).[20,23−26] Likewise, the cost of platelet transfusions has increased recently due to a trend toward administration of single-donor platelet packs over multiple-donor combination packs.[27,28] Therefore, a low-cost, safe thrombopoietic agent which can lessen or eliminate the need for platelet transfusions (and thereby also lower the frequency of platelet apheresis from healthy donors) would benefit patient health and also significantly lower health-care costs.

The only approved platelet growth factor available at this time is the protein therapeutic rhuIL-11 (Neu-

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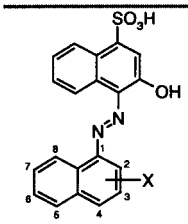
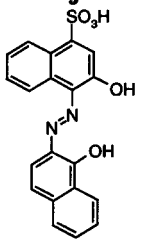
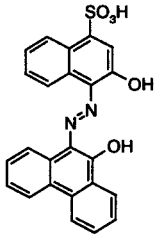
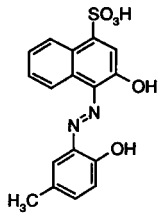
mega),[29] which is approved for prevention of severe thrombocytopenia and the reduction of the need for platelet transfusions following myelosuppressive chemotherapy in patients with nonmyeloid malignancies who are at high risk of severe thrombocytopenia. rhuTPO[30] and pegylated megakaryocyte differentiation factor[31] (peg-MGDF, a pegylated, truncated, N-terminal region of TPO) are two protein agents currently undergoing clinical trials for the treatment of thrombocytopenia. Although recombinant hematopoietic growth factors such as rhuEPO (Epogen) and rhuG-CSF (Neupogen) have proven to be successful prescribed medications, there is a great opportunity for a small-molecule, nonpeptidyl, orally administered agent in this area. The obvious benefits are a lower cost of production and an easier route of administration (ideally orally) with the concomitant benefits of outpatient treatment and nonimmunogenicity. That such an approach was feasible was first demonstrated by the discovery of the nonpeptidyl mimic of granulocyte-colony-stimulating factor (G-CSF), SB-247464.[32]

Results and Discussion

Development of a High-Throughput Screen for TPO Mimics. Our search for a nonpeptidyl thrombopoietic agent began with the development of an assay suitable for rapid screening of several hundred thousand compounds. To this end a high-throughput, cell-based assay was developed to detect compounds that activate human TPO receptor (hTPOr) linked signal transduction pathways. This assay utilizes a reporter gene encoding the protein luciferase,[33,34] the expression of which is driven by a synthetic STAT-responsive promoter that is stably transfected into a TPO-responsive cell line.[35−37] TPO treatment of these cells results in activation of STAT5, which binds to the synthetic promoter and increases luciferase expression. The murine hematopoietic progenitor cell line BAF-3 was transfected with both cloned hTPOr cDNA and a luciferase reporter construct. Screening of a large number of stably transfected, drug-resistant clones resulted in the identification of a suitable TPO-responsive cell line, designated BAF-3/TPO-Rluc. A dose response in the BAF-3/TPO-Rluc cell line with respect to TPO consistently afforded a 15−20-fold maximal increase in luciferase production (typically at [TPO] ≈ 0.1 ng/mL) with EC_{50} = 0.001−0.01 ng/mL. This large window gave us the opportunity to identify even relatively weak activators of the TPO signaling pathway.

Luciferase Assay Structure−Activity Relationships (SARs). Screening of the SmithKline Beecham and Ligand proprietary compound collections against the BAF-3/TPO-Rluc cell line resulted in the discovery of compound **2a** with a reproducible EC_{50} = 0.2 μM and 38% efficacy (compared to that of maximal [TPO]). Deep purple diazonaphthalene **2a** is representative of a class of compounds historically used in the dyestuffs industry, Eriochrome Blue Black R (C.I. 15705), often used as a colorimetric indicator for metal ions.[13,38,39] Although compound **2a** contains unattractive and potentially reactive functionality, the consistent activity of the compound in our selectivity and cell-signaling assays prompted investigations into the initial SAR in the luciferase assay around both naphthalene rings. As shown in Table 1, a series of dihydroxy compounds **2b**−**e** maintaining the 2-hydroxyl were prepared, all of which were active in the luciferase assay albeit with at least a 10-fold loss in potency. Removing the 2-hydroxy group (compound **2f**) resulted in a loss in activity, although a methoxy group at the 2-position was better tolerated (compound **2g**). The addition of a 7-methoxy group to the original lead **2a** (compound **2h**) was tolerated and gave an improvement in efficacy, although inclusion of a carboxylic acid was less well tolerated (compounds **2i** and **2j**). The 1-hydroxy-2-naphthyl regioisomer **3** was slightly more potent (0.07 μM) than **2a** and significantly more efficacious (87% TPO). While 9-hydroxyphenanthrene **4** was nearly equivalent in potency and efficacy compared to **2a**, the 2-hydroxy-5-methylphenyl analogue

Table 1. BAF-3/TPO Luciferase Activity: Azonaphthols. SAR in the Lower Naphthalene Ring

	X	Efficacy (%TPO)	EC_{50} (μM)
			
2a	2-OH	38	0.2
2b	2,3-diOH	26	2
2c	2,4-diOH	16	5
2d	2,6-diOH	16	2
2e	2,7-diOH	31	2
2f	4.7-diOH	<5	-
2g	2-OMe, 4-OH	19	3
2h	2-OH, 7-OMe	85	0.3
2i	2-OH, 3-CO_2H	48	10
2j	2-OH, 5-CO_2H	<5	-
 **3**	-	87	0.07
 **4**	-	61	0.2
 **5**	-	28	3

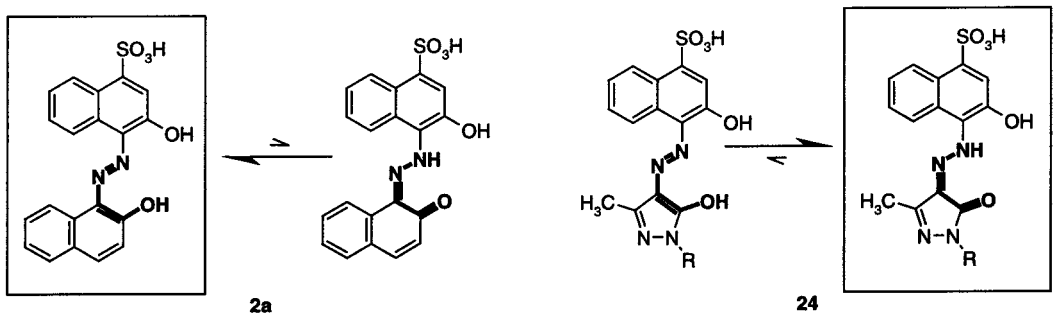
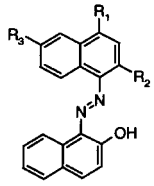


Figure 1. Comparison of preferred tautomeric equilibria of hydroxynaphthalene screening hit **2a** with pyrazolin-5-ones **24.**

Table 2. BAF-3/TPO Luciferase Activity: Azonaphthols. SAR in the Upper Naphthalene Ring



	R_1	R_2	R_3	Efficacy (%TPO)	EC_{50} (μM)
1a	SO_3H	OH	H	38	0.2
8a	SO_3H	H	H	<5	-
8b	H	OH	H	<5	-
8c	NO_2	OH	H	<5	-

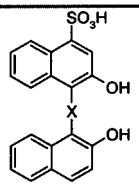
5 was significantly weaker in both respects. These data suggest the need for an electron-donating group and/or hydrogen bond acceptor at position 2 of the lower ring and that further addition of polar groups (OH/CO_2H) in the lower ring is detrimental.

Several specific modifications in the upper naphthalene ring of compound **2a** were also investigated (Table 2). The presence of both the 2-hydroxyl group and the 4-sulfonic acid was found to be important since removal of either (compounds **8a** and **8b**, respectively) or replacement of the sulfonic acid with a nitro group (compound **8c**) resulted in a dramatic drop in activity.

With definite SAR trends emerging, compound **2a** was emerging as a potential lead. On the other hand, there were a number of issues with the structure of this class of compounds which presented clear problems for further development. The foremost issue was the possibility of azoreduction in vivo, particularly by intestinal bacteria,[40] resulting in the destruction of the molecule prior to absorption. In addition to a loss in active compound, such a transformation would result in the production of lipophilic aminonaphthols, a compound class which contains known mutagens. Replacement of the diazo group with two-atom isosteres (Table 3) led to the imine **10a**, aminomethylene **10b**, and amide **10c**, which proved to be detrimental to activity in the luciferase assay.

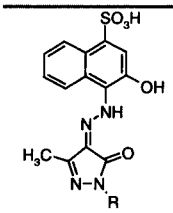
An alternative approach to obviating the azoreduction liability was again suggested by precedents from the dyestuffs literature. Orange food coloring tartrazine is an azopyrazole which is known to be resistant to azoreduction both in vivo and in vitro.[42] It is postulated that this resistance is due to its existence solely as the (5-oxo-pyrazol-4-ylidene)hydrazine tautomer, which is not recognized by the reductase enzymes.[43−49] Therefore, replacement of the lower naphthol ring of our diazonaphthol compounds with various pyrazolin-5-ones was

Table 3. BAF-3/TPO Luciferase Activity: Isosteric Replacement of Diazo Functionality



	X	Efficacy (%TPO)	EC_{50} (μM)
10a		<5	-
10b		<5	-
10c		<5	-

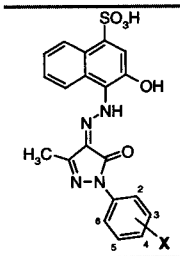
Table 4. BAF-3/TPO Luciferase Activity: Pyrazol-4-ylidenehydrazines. N-Substituent SAR



	R	Efficacy (%TPO)	EC_{50} (μM)
24a	H	<5	-
24b	CH_3	<5	-
24c	t-Bu	<5	-
24d	Ph	26	2
24e	pyridin-2-yl	24	7
24f	benzyl	32	>30
24g	2-hydroxyethyl	<5	-

undertaken (Figure 1) in the hope that potency would be retained and the potential for azoreduction eliminated. Fortunately, the pyrazolinone was an effective replacement for the naphthol group with activity retained albeit at somewhat reduced potency (Table 4). The SAR of the pyrazolinones was very sensitive to the nature of the N-substituent, with unsubstituted compound **24a**, alkyl compounds **24c,b**, and substituted alkyl compound **24g** being poor agonists in the luciferase assay**.** From this group the 1-phenyl analogue **24d** was the most active ($EC_{50} = 2\ \mu M$), with the 2-pyridinyl and benzyl analogues **24e** and **24f** being significantly less potent. Encouraged by these results, we undertook the synthesis and screening of a series of substituted 1-aryl analogues (Table 5). All possible monochloro, mononitro,

Table 5. BAF-3/TPO Luciferase Activity: Pyrazol-4-ylidenehydrazines. 1-Phenyl SAR



	X	Efficacy (%TPO)	EC_{50} (μM)
24d	H	26	2
25a	2-Cl	6	>30
25b	3-Cl	34	2
25c	4-Cl	47	2
25d	2-NO_2	19	8
25e	3-NO_2	28	0.3
25f	4-NO_2	63	0.4
25g	2-CH_3	30	10
25h	3-CH_3	34	0.7
25i	4-CH_3	53	2
25j	3-CF_3	62	0.04
25k	4-CF_3	39	0.1
25l	4-F	39	0.5
25m	4-I	81	0.3
25n	4-OCH_2Ph	34	0.2
25o	3,4-diCl	67	0.2
25p	3,4-diCH_3	100	0.04
25q	4-$CH(CH_3)_2$	100	0.07
25r	3-$C(CH_3)_3$	71	0.07
25s	4-$C(CH_3)_3$	86	0.02
25t	4-SO_2CH_3	18	3
25u	3-$SO_2NHCH_2CH_3$	25	10
25v	4-CO_2H	<5	-
25w	4-SO_3H	<5	-
25x	3-SO_3H, 6-Cl	<5	-
25y	4-SO_3H, 2,5-diCl	<5	-
25z	4-OH	<5	-

and monomethyl analogues were prepared (**25a**−**i**) with the following SAR trends emerging: 2-substitution was always detrimental to activity, 3- and 4-monochloro and 3- and 4-monomethyl substitution were approximately equipotent to the unsubstituted phenyl compound **24d**, and 3- and 4-mononitro substitution gave an approximate 6-fold increase in potency. The 3-trifluoromethyl analogue **25j** showed a marked increase in potency (EC_{50} = 0.04 μM), with its corresponding 4-regioisomer being about 2-fold weaker. Significantly, the full efficacy brought about by saturating concentrations of TPO in the luciferase assay was achieved by incorporating bulkier lipophilic groups in the *N*-phenyl ring. For example, 3,4-dimethylphenyl analogue **25p** (EC_{50} = 0.04 μM, 100% TPO; Figure 2) and 4-isopropyl analogue **25q** (EC_{50} = 0.07 μM, 100% TPO) were as efficacious as TPO itself and exhibited low nanomolar potencies. In a fashion similar to that of the naphthalene series, substitution in the phenyl ring with polar groups was not well tolerated as shown by the loss of activity in benzoic acid **25v**, sulfonic acids **25w**−**y**, and phenol **25z**.

A series of compounds was next prepared by substituting the pyrazole at the 3-position (Table 6) while

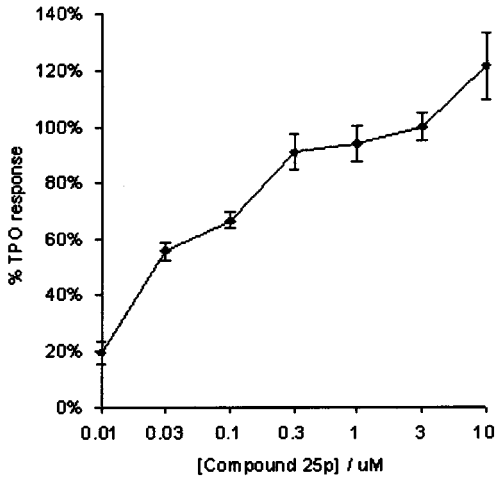
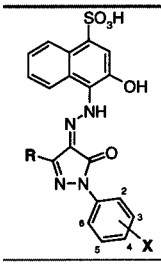


Figure 2. BAF-3/TPO luciferase assay for compound **25p**. BAF3-3B5/TPO cells (1×10^5/mL) (starved of IL-3 overnight) in growth medium containing FBS (0.5% v/v) and $ZnCl_2$ (30 μM) were incubated with compounds (0.32% DMSO final concentration) or rhTPO at 37 °C (5% CO_2, 95% relative humidity) for 3 h. Luciferase activity was recorded using a Dynatech model 1000 luminometer. Each data point is the average of triplicate assays.

Table 6. BAF-3/TPO Luciferase Activity: Pyrazol-4-ylidenehydrazines. SAR at the C-3 Position

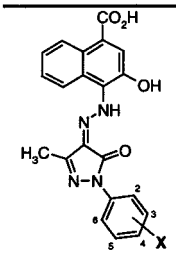


	R	X	Efficacy (%TPO)	EC_{50} (μM)
25p	CH_3	3,4-diCH_3	100	0.04
26a	H	3,4-diCH_3	95	0.1
26b	$C(CH3)_3$	3,4-diCH_3	72	0.3
26c	Ph	3,4-diCH_3	92	0.9
26d	OCH_2CH_3	3,4-diCH_3	80	0.6
26e	$CO_2CH_2CH_3$	4-$C(CH_3)_3$	56	2
26f	CO_2 $C(CH3)_3$	4-$C(CH_3)_3$	32	0.2
26g	CO_2H	4-$C(CH_3)_3$	<5	-
26h	NH_2	4-$C(CH_3)_3$	87	5

maintaining the 1-aryl ring as 3,4-dimethylphenyl or 4-*tert*-butylphenyl. Removal of the methyl group altogether afforded compound **26a,** which retained potency, while substitution with bulkier groups such as *tert*-butyl (compound **26b**) and phenyl (compound **26c**) gave significantly weaker compounds. The ethyl ether analogue **26d** showed approximately a 10-fold reduction in potency. Likewise, both the ethyl ester **26e** and the *tert*-butyl ester **26f** showed concomitant potency losses. Polar groups such as a carboxylic acid (**26g**) or amine (**26h**) at this position were poorly tolerated.

Finally, compounds in which the sulfonic acid was replaced with a carboxylic acid (Table 7) (compounds **30a**−**c**) were significantly less potent and efficacious when compared with the sulfonic acid analogues, indicating that a sulfonic acid is preferred at the 4-position of the naphthalene ring.

Table 7. BAF-3/TPO Luciferase Activity: Naphthalene-1-carboxylic Acids



	X	Efficacy (%TPO)	EC_{50} (μM)
30a	H	21	5
30b	4-CH_3	47	2
30c	3,4-diCH_3	24	1

Table 8. Luciferase Activity of Various Cytokine-Responsive Cell Lines to Compound **25p**

cell line	cytokine response	positive control	TPO	compound **25p**
BAF3/TPO	IL-3/TPO	+	+	+
UT7/TPO	TPO	+	+	+
hMPL-HepG2	TPO	+	+	+
BAF3 parental	IL-3	+	−	−
BAF3/G-CSF	G-CSF	+	−	−
NFS-60	G-CSF	+	−	−
Kasumi 1	G-CSF	+	−	−
TF-1	GC-CSF/EPO	+	−	−
UT7/EPO (ENDB7)	EPO	+	−	−

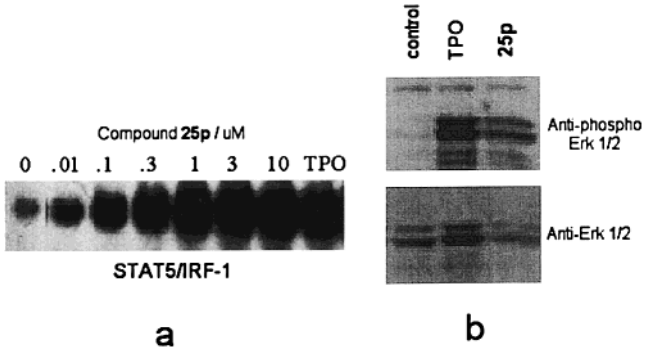


Figure 3. Activation of (a) STAT-5 phosphorylation and (b) MAPK in BAF3/TPO cells for compound **25p**.

Further Biological Characterization of Pyrazol-4-ylidenehydrazines. In addition to the luciferase assay, active analogues were tested in a panel of secondary assays to assess their potential as true thrombopoietin mimics. In a series of luciferase assays utilizing various cytokine-responsive cell lines, compound **25p** was found to be active only in cells expressing hTPOr (Table 8). In TPO-responsive cell lines, compound **25p** promoted DNA binding by STAT5[37] as judged using an electrophoretic mobility shift assay (EMSA) (Figure 3a) and induced phosphorylation of MAPK,[9] detected by Western blot using an antibody specific for the phosphorylated form of ERK1-2 (Figure 3b).

To further test the thrombopoietic activity, compounds were tested for their ability to support proliferation of the UT7/TPO cell line. As shown in Table 9, there was an excellent correlation in the SAR between proliferation and luciferase assays, therefore validating the latter assay as a tool for rapid SAR evaluation of analogues. In general the efficacies and potencies proved

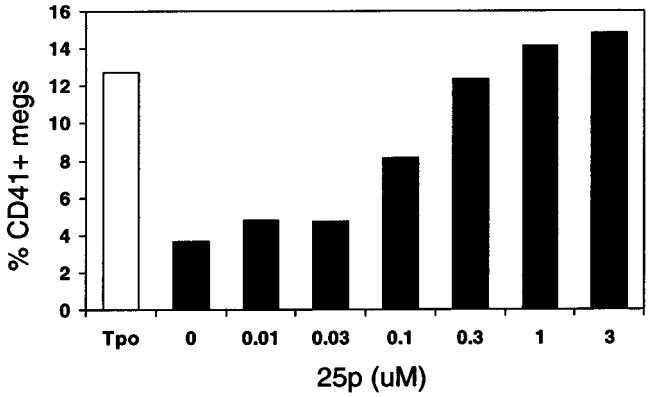


Figure 4. $CD41^+$ assay for compound **25p**. Human bone marrow $CD34^+$ cells (2×10^6 cells/mL) in IMDM containing fetal calf serum (20% v/v) and recombinant human stem cell factor (100 ng/mL) were incubated with compound **25p** for 10 days at 5% CO_2 and 4% O_2. Samples were stained with FITC-anti-IgG1 isotype control and FITC-anti-CD41 (Pharmingen clone SZ22) and fixed in formaldehyde, and the cells were analyzed on a Becton-Dickinson FACScan flow cytometer. The percent of CD41 cells for each sample was calculated by subtracting the isotype control positive from the CD41 positive cells.

to be slightly better in the longer proliferation assay with compounds containing lipophilic groups in the N-1 phenyl ring, such as the 4-*tert*-butyl compound analogue **25s** showing a reproducible EC_{50} as low as 0.001 μM in the UT7/TPO proliferation assay with full TPO efficacy.

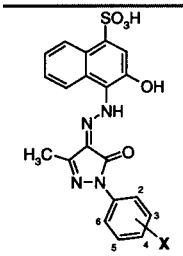
In addition to a proliferative effect on cells of the megakaryocytic lineage, TPO also induces differentiation of hematopoietic stem cells into committed megakaryocyte progenitors. Assessment of megakaryocyte maturation can be effected by measuring the appearance of the megakaryocyte-specific marker glycoprotein CD41 on human $CD34^+$ cells obtained from bone marrow samples. In this assay only cells committed to the megakaryocyte lineage express CD41, a heterodimeric, MW 140000, transmembrane glycoprotein also known as platelet glycoprotein GPIIb, which is the α subunit of the integrin $\alpha_{IIb\beta3}$. Table 9 shows the activities of selected analogues in this differentiation assay showing potencies as low as 0.03 μM and efficacies again as high as that of saturating TPO concentrations. Figure 4 exemplifies a dose−response counting the percentage of human bone marrow $CD34^+$ cells cultured in suspension expressing the CD41 marker after a 10 day incubation period for the 3,4-dimethyl analogue **25p**. In this assay, compound **25p** exhibits at least the full efficacy of TPO with an approximate $EC_{50} \approx 0.2$ μM.

Therefore, the pyrazol-4-ylidenehydrazines described herein elicit a profile of biological responses identical to those of TPO itself. Studies to elucidate the mechanism of interaction of the pyrazol-4-ylidenehydrazines with the TPO receptor, whether the compounds cause receptor oligomerization, therefore mimicking a cytokine physically as well as biologically, are ongoing.

Chemistry

Diazo Analogues. The synthesis of 3-hydroxy-4-azonaphthalene-1-sulfonic acids **2a**−**j** and **3**−**5** (Scheme 1) is carried out using commercially available 4-diazo-3-hydroxynaphthalene-1-sulfonic acid in a coupling reaction with the requisite naphthol, phenol, or phenan-

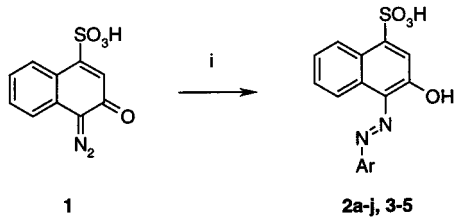
Table 9. UT7/TPO Cell Proliferation Data and $CD34^+$ Cell Differentiation Data for Selected Pyrazol-4-ylidenehydrazines[a]



X	Luciferase		Proliferation		Differentiation	
	Efficacy (%TPO)	EC_{50} (μM)	Efficacy (%TPO)	EC_{50} (μM)	Efficacy (%TPO)	EC_{50} (μM)
25b 3-Cl	34	2	45	0.5	-	-
25c 4-Cl	47	2	75	0.5	-	-
25i 4-CH_3	53	2	90	0.5	-	-
25j 3-CF_3	62	0.04	120	0.07	95	0.1
25k 4-CF_3	39	0.1	100	0.05	-	-
25m 4-I	81	0.3	100	0.1	60	0.02
25o 3,4-diCl	67	0.2	70	0.05	-	-
25p 3,4-diCH_3	100	0.04	90	0.04	100	0.2
25s 4-$C(CH_3)_3$	86	0.02	100	0.001	100	0.03

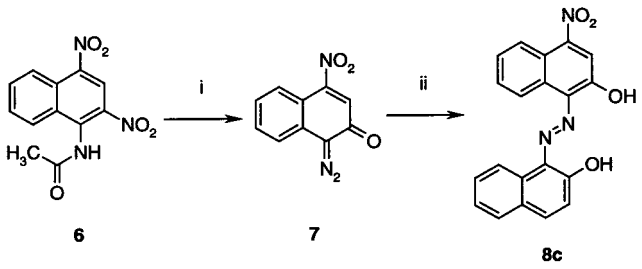
[a] The "-" means not tested.

Scheme 1. Synthesis of Diazonaphthalenes **2a**−**j** and **3**−**5**[a]



[a] Reagents and conditions: (i) appropriate naphthol, phenol, or phenanthrol, $NaHCO_3$, H_2O, ethanol, 60 °C.

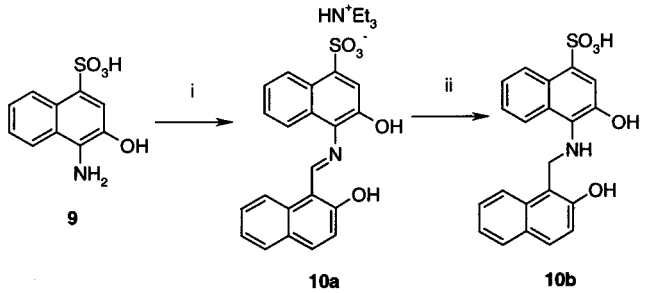
Scheme 2. Synthesis of Nitronaphthol **8c**[a]



[a] Reagents and conditions: (i) concentrated H_2SO_4, water, and then $NaNO_2$, concentrated H_2SO_4, 0 °C; (ii) 2-naphthol, $NaHCO_3$, H_2O, 60 °C.

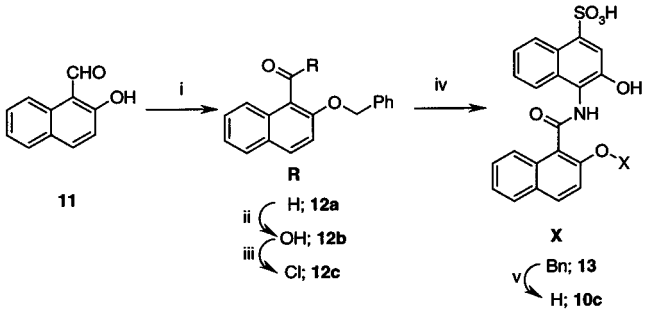
throl. Water or aqueous ethanol buffered with excess sodium hydrogen carbonate at 60 °C was found to be the optimum reaction condition for the highest yields, purity of products, and simplicity of product isolation. Couplings were typically run overnight to ensure complete reaction. The 2-unsubstituted diazo compound **8a** was prepared by diazotization of 4-aminonaphthalene-1-sulfonic acid followed by coupling with 2-naphthol. Symmetrical azonaphthol **8b** was prepared from 2-naphthol sodium salt and *p*-toluenesulfonyl azide by a literature method.[50] The synthesis of 4-nitro-2-hydroxy-1-azo analogue **8c** (Scheme 2) involves hydrolysis followed by diazotization of commercially available 1-acetamido-2,4-dinitronaphthalene (**6**) to give the diazonium species **7** according to a literature method.[51] Subsequent coupling with 2-naphthol as previously described affords the desired diazo compound **8c**.

Scheme 3. Synthesis of Aminomethylene Analogue **10b**[a]



[a] Reagents and conditions: (i) 2-hydroxy-1-naphthaldehyde, Et_3N, EtOH, relfux, 60 °C; (ii) BH_3·THF, THF, −78 °C to rt.

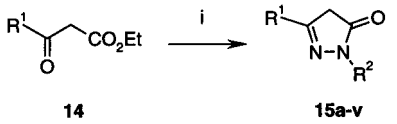
Scheme 4. Synthesis of Amide **10c**[a]



[a] Reagents and conditions: (i) $PhCH_2Cl$, K_2CO_3, ethanol, reflux; (ii) $NaClO_2$, NH_2SO_3H, acetone, H_2O, 0 °C; (iii) $(COCl)_2$, DMF, toluene, rt; (iv) 4-amino-3-hydroxy-1-naphthalenesulfonic acid, pyridine, benzene, reflux; (v) H_2, 10% Pd−C, MeOH.

Isosteric Replacements for the Diazo Group. The imine **10a** (Scheme 3) was synthesized by refluxing an ethanolic solution of the amine **9** and 2-hydroxy-1-naphthaldehyde (**11**) in the presence of triethylamine.[52] Reduction of imine **10a** to aminomethylene compound **10b** was achieved with the borane−THF complex. To prepare amide analogue **10c** (Scheme 4), hydroxyaldehyde **11** was protected as its benzyl ether **12a** and then oxidized to the known acid[53] **12b** using sodium chlorite.

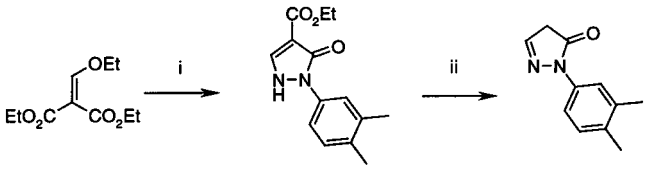
Scheme 5. Synthesis of Pyrazol-3-ones **15a**−**v**[a]



14 **15a-v**

[a] Reagents and conditions: (i) R_2NHNH_2·HCl, NaOAc, AcOH, reflux.

Scheme 6. Synthesis of Pyrazol-3-one **18**[a]



16 **17** **18**

[a] Reagents and conditions: (i) $ArNHNH_2$·HCl, K_2CO_3, EtOH, reflux; (ii) NaOH and then HCl.

The acid was converted to the acid chloride **12c** and was subsequently coupled with the amine **9** to afford the protected amide **13** in low yield. Final hydrogenation removed the benzyl protecting group, affording amide isostere **10c**.

Pyrazol-4-ylidenehydrazine Analogues. The many 4-unsubstituted 2,4-dihydropyrazol-3-ones (C.A. Index numbering) **15a**−**v, 18, 21,** and **23** used in this work were either commercially available or prepared by a number of standard methods. 2,5-Disubstituted pyrazolin-3-ones **15a**−**v** were prepared (Scheme 5) by the condensation of the appropriate β-ketoester **14** with the corresponding substituted hydrazine in refluxing acetic acid**.** 5-Unsubstituted pyrazolin-3-one **18** was prepared (Scheme 6) by the condensation of 2-(ethoxymethylene)-malonic acid diethyl ester (**16**) with 3,4-dimethylphenylhydrazine to afford 3-(carboxyethyl)pyrazolone **17** followed by ester hydrolysis and thermal decarboxylation. To prepare 5-ethoxypyrazolin-3-one **21** (Scheme 7), *N*′-acetyl-3,4-dimethylphenylhydrazine (**19**) was condensed with malonic acid in the presence of phosphorus trichloride to afford pyrazoline-3,5-dione **20**, which then underwent acid-catalyzed condensation with ethanol to give ethoxy analogue **21**. 5-Aminopyrazolin-3-one **23** was straightforwardly prepared (Scheme 8) from ethyl cyanoacetate and phenylhydrazine **22**. Coupling of these pyrazolin-3-ones with diazo compound **1** in the presence of sodium hydrogen carbonate in aqueous ethanol at 60 °C afforded the requisite pyrazol-4-ylidenehydrazines **24a**−**g**, **25a**−**z**, and **26a**−**h** (Scheme 9) in excellent yield and purity in the majority of cases.

Naphthalenecarboxylic Acids 30. 3-Hydroxy-1-naphthalenecarboxylic acid (**28**) was prepared (Scheme 10) from 3-nitro-1,8-naphthoic anhydride (**27**) according to literature procedures.[54,55] Coupling of naphthol **28** with *p*-benzenediazonium sulfonate followed by reduction afforded 4-amino compound **29,** which was then diazotized and coupled with representative pyrazolinones to afford analogues **30a,b**. An alternative and higher yielding procedure to prepare analogue **30c** involves first treatment of the pyrazolinone with tosyl azide, effecting a diazo transfer to the pyrazole. The resulting 4-diazopyrazolinone was then coupled with naphthol **28** to give the pyrazol-4-ylidenehydrazine **30c**.

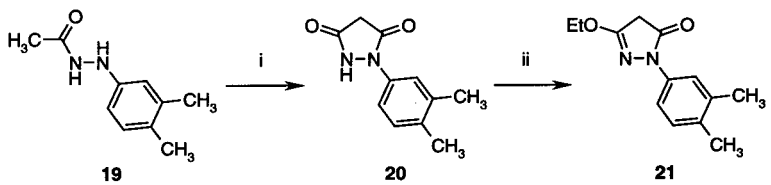
Conclusion

In this study we report the discovery of low molecular weight, nonpeptidyl TPO mimics. These compounds have activity comparable to that of TPO in cells of the megakaryocyte lineage in all assays tested. They activate JAK and MAP kinases, promote the phosphorylation of STAT5, and promote the proliferation of several TPO-responsive cell lines. Most importantly, these mimics stimulate the differentiation of human $CD34^+$ bone marrow cells into megakaryocyte precursors in a dose-responsive manner, therefore acting on the relevant target human tissue. Chemistry was optimized to provide these TPO mimics in relatively few steps (usually only two steps required) from commercial reagents in high chemical yields and purities. The activities for the best compounds were remarkable for such low molecular weight compounds (MW < 500) with EC_{50} values in the region of 1−20 nM in cell proliferation and differentiation assays with efficacies comparable with that of TPO itself. Preliminary pharmacokinetic analysis of the more potent naphthalenesulfonic acid containing pyrazol-4-ylidenehydrazines revealed very low to no oral absorption for these polar compounds in both rodent and nonrodents species presumably due to the hydrophilicity of the ionized sulfonate group. The introduction of oral bioavailability into the pyrazol-4-ylidenehydrazines by the successful discovery of sulfonic acid surrogates (without the concomitant loss in TPO agonist activity as seen with the naphthalene carboxylic acids **30a**−**c**, vide supra) will be the subject of future publications.

Experimental Section

Chemistry. General Methods. All starting materials were either commercially available or prepared as reported previously in the literature unless noted. Solvents and reagents were used without further purification, except THF, which was dried over sodium. Reactions were monitored by TLC on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). Purification was performed by flash chromatography using silica gel (particle size 40−63 mesh, Merck). ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 instrument. Chemical shifts are reported as parts per million relative to TMS as internal standard in the solvent indicated. Mass spectra were recorded on a Micromass electrospray LC−mass spectrometer. IR spectra were recorded on a Nicolet 510 FT-IR spectrometer. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Microanalysis was carried out

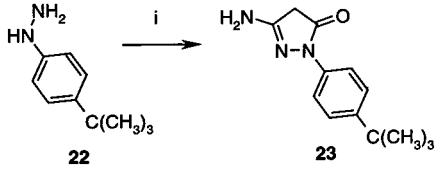
Scheme 7. Synthesis of 5-Ethoxypyrazol-3-one **21**[a]



19 **20** **21**

[a] Reagents and conditions: (i) malonic acid, $POCl_3$, reflux; (ii) concentrated H_2SO_4, EtOH.

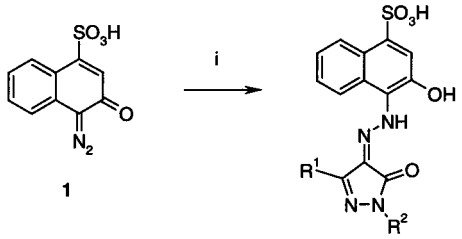
Scheme 8. Synthesis of 5-Aminopyrazol-3-one **23**[a]



22 23

[a] Reagents and conditions: (i) ethyl cyanoacetate, NaOEt, EtOH, reflux.

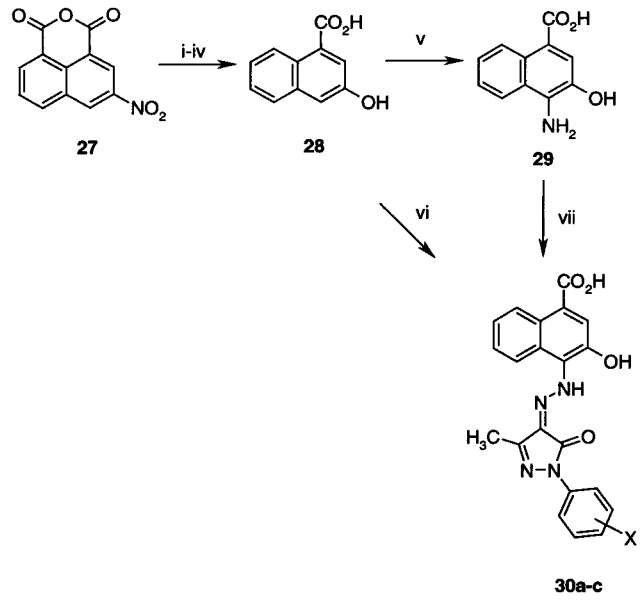
Scheme 9. Synthesis of Pyrazol-4-ylidenehydrazines **24a**−**g**, **25a**−**z**, and **26a**−**i**[a]



1 **24a-g, 25a-z, 26a-i,**

[a] Reagents and conditions: (i) pyrazolones **15**, $NaHCO_3$, H_2O, ethanol, 60 °C.

Scheme 10. Synthesis of Naphthalene-4-carboxylic Acids **30a**−**c**[a]



27 28 29 **30a-c**

[a] Reagents and conditions: (i) HgO, NaOH, H_2O, reflux; (ii) concentrated HCl, H_2O, reflux; (iii) H_2, 10% Pd−C, NaOH, EtOH, H_2O; (iv) $NaNO_2$, H_2SO_4, H_2O, 10 °C, and then 40% aqueous H_2SO_4, reflux; (v) *p*-diazoniumbenzenesulfonamide, $NaHCO_3$, H_2O then $NaHSO_3$, 60 °C; (vi) pyrazolinone **15m**, TsN_3, Et_3N, and then Et_3N, EtOH; (vii) pyrazolinone **15m**, $NaHCO_3$, H_2O, ethanol, 60 °C.

by QTI Technologies, Inc., Whitehouse, NJ. Sulfonic and carboxylic acids were produced either as sodium salts as precipitates from the slightly basic reaction media or as free acids after acidification and reprecipitation to give compounds of >95% purity as detected by reversed-phase HPLC and ^{1}H NMR. Sulfonic acid analogues were normally isolated as hydrates containing between 0.25 and 3 waters of hydration. Variation in sulfonate salt form had a negligible effect on biological activity.

General Method for the Synthesis of Diazo Analogues 2−5.

3-Hydroxy-4-[(2-hydroxy-1-naphthalenyl)azo]-1-naphthalenesulfonic Acid (2a). Solid sodium hydrogen carbonate (1.50 g, 0.018 mol) was added slowly to a stirred solution of 1-diazo-2-naphthol-4-sulfonic acid (Aldrich Chemical Co.) (1.49 g, 5.95 mmol) and 2-naphthol (0.858 g, 5.95 mmol) in water (20.0 mL) and ethanol (20.0 mL) at room temperature. The resulting solution was heated and stirred at 60 °C overnight. The solution was cooled to room temperature, and was adjusted to pH 1 with 3 M aqueous hydrochloric acid. The purple precipitate was isolated by filtration and washed with water to provide the title compound (1.35 g, 58%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 14.3 (s, 1H), 13.8 (s, 1H), 8.84 (t, J = 8.4 Hz, 2H), 8.76 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.85−7.89 (m, 2H), 7.72 (s, 1H), 7.53−7.47 (s, 2H), 7.23 (d, J = 9.2 Hz, 1H).

4-[(2,3-Dihydroxy-1-naphthalenyl)azo]-3-hydroxy-1-naphthalenesulfonic Acid (2b). Following the procedure of **2a**, except substituting 2,3-dihydroxynaphthalene for 2-naphthol, the title compound was prepared (2.24 g, 70%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 16.4 (s, 1H), 12.1 (s, 1H), 9.17 (d, J = 8.6 Hz, 1H), 8.90 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.71 (t, J = 7.3 Hz, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.51−7.44 (m, 2H), 7.37 (t, J = 8.0 Hz, 1H), 7.18 (s, 1H).

4-[(2,4-Dihydroxy-1-naphthalenyl)azo]-3-hydroxy-1-naphthalenesulfonic Acid (2c). Following the procedure of **2a**, except substituting 2,4-dihydroxynaphthalene for 2-naphthol, the title compound was prepared (2.11 g, 87%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 16.6 (s, 1H), 13.3 (br s, 1H), 9.02 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.86 (s, 1H), 7.68−7.60 (m, 2H), 7.49−7.44 (m, 2H), 6.21 (s, 1H).

4-[(2,6-Dihydroxy-1-naphthalenyl)azo]-3-hydroxy-1-naphthalenesulfonic Acid (2d). Following the procedure of **2a**, except substituting 2,6-dihydroxynaphthalene for 2-naphthol, the title compound was prepared (2.03 g, 71%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 14.0 (s, 1H), 13.8 (s, 1H), 9.8 (br s, 1H), 8.84 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.5 Hz, 1H), 8.63 (d, J = 9.0 Hz, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.72−7.67 (m, 1H), 7.70 (s, 1H), 7.48 (t, J = 8.2 Hz, 1H), 7.29 (m, 1H), 7.19−7.14 (m, 2H).

4-[(2,7-Dihydroxy-1-naphthalenyl)azo]-3-hydroxy-1-naphthalenesulfonic Acid (2e). Following the procedure of **2a**, except substituting 2,7-dihydroxynaphthalene for 2-naphthol, the title compound was prepared (2.13 g, 78%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 14.6 (s, 1H), 13.6 (s, 1H), 10.3 (s, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.80 (d, J = 8.5 Hz, 1H), 8.00 (d, J = 2.1 Hz, 1H), 7.86 (d, J = 9.1 Hz, 1H), 7.76 (s, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.70 (td, J = 8.1 and 2.1 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.00 (dd, J = 8.7 and 2.2 Hz, 1H), 6.96 (d, J = 9.1 Hz, 1H).

4-[(4,7-Dihydroxy-1-naphthalenyl)azo]-3-hydroxy-1-naphthalenesulfonic Acid (2f). Following the procedure of **2a**, except substituting 4,7-dihydroxynaphthalene for 2-naphthol, the title compound was prepared (2.13 g, 78%) as a purple solid: ^{1}H NMR (d_6-DMSO) δ 15.7 (s, 1H), 11.1 (s, 1H), 8.86 (d, J = 8.3 Hz, 1H), 8.80 (d, J = 8.3 Hz, 1H), 8.21 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 9.1 Hz, 1H), 7.65 (s, 1H), 7.64 (m, 1H), 7.49 (t, J = 7.3 Hz, 1H), 7.15 (dd, J = 9.1 and 2.1 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H).

3-Hydroxy-4-[(4-hydroxy-2-methoxy-1-naphthalenyl)-azo]-1-naphthalenesulfonic Acid (2g). (a) 3-Methoxy-1-naphthol. To a stirring solution of 1,3-dihydroxynaphthalene (2.31 g, 14.4 mmol) in methanol (150 mL) at 0 °C was bubbled hydrochloride gas for 10 min. The resulting solution was stirred at room temperature for 24 h. A residue was obtained after evaporation of methanol and purified by silica gel column chromatography eluted with chloroform to provide the title compound as purple crystals (1.42 g, 57%): ^{1}H NMR ($CDCl_3$) δ 8.08 (d, 1H), 7.70 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 6.78 (s, 1H), 6.54 (s, 1H), 5.61 (s, 1H), 3.90 (s, 3H).

(b) 3-Hydroxy-[4-(4-hydroxy-2-methoxy-1-naphthalenyl)azo]-1-naphthalenesulfonic Acid (2g). Following the procedure of **2a**, except substituting 3-methoxy-1-naphthol for 2-naphthol, the title compound was prepared as a purple solid (2.37 g, 88%): ^{1}H NMR (d_6-DMSO) δ 16.1 (br s, 1H), 11.8 (br s, 1H), 9.6 (br s, 1H), 8.82 (d, J = 8.5 Hz, 1H), 8.71 (dd, J =

8.3 Hz, 1H), 8.23 (m, 1H), 7.82 (m, 1H), 7.70−7.60 (m, 2H), 7.52−7.41 (m, 3H), 7.68 (m, 1H), 4.12 (s, 3H).

3-Hydroxy-4-[(2-hydroxy-7-methoxy-1-naphthalenyl)-azo]-1-naphthalenesulfonic Acid (2h). Following the procedure of **2a**, except substituting 7-methoxy-2-naphthol for 2-naphthol, the title compound was prepared as a black solid (2.66 g, 74%): ^{1}H NMR (d_6-DMSO) δ 14.1 (s, 1H), 13.8 (s, 1H), 8.86 (td, J = 9.0 and 1.2 Hz, 1H), 8.35 (d, J = 2.3 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.71 (s, 1H), 7.65 (td, J = 8.0 and 1.2 Hz, 1H), 7.48 (td, J = 7.8 and 1.2 Hz, 1H), 7.13 (dd, J = 8.8 and 2.5 Hz, 1H), 4.01 (s, 3H).

3-Hydroxy-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)azo]-naphthalene-2-carboxylic acid (2i) was purchased from Aldrich Chemical Co., Milwaukee, WI, and used as provided.

3-Hydroxy-4-[(2-hydroxy-5-methylphenyl)azo]naphthalene-1-sulfonic Acid (2j). Following the procedure of **2a**, except substituting 6-hydroxynaphthalene-1-carboxylic acid for 2-naphthol, the title compound was prepared as a dark brown solid (3.0 g, 72%): ^{1}H NMR (d_6-DMSO) δ 14.6 (br s, 1H), 13.4 (br s, 1H), 8.96 (d, J = 8.3 Hz, 1H), 8.86 (d, J = 9.0 Hz, 2H), 8.06 (d, J = 7.3 Hz, 1H), 7.83−7.69 (m, 3H), 7.50 (t, J = 8.3 Hz, 1H), 7.37 (d, J = 9.6 Hz, 1H).

3-Hydroxy-4-[(1-hydroxy-2-naphthalenyl)azo]-1-naphthalenesulfonic acid (3) was purchased from Aldrich Chemical Co., Milwaukee, WI, and used as provided.

3-Hydroxy-4-[(10-hydroxy-9-phenanthrenyl)azo]-1-naphthalenesulfonic Acid (4). Following the procedure of **2a**, except substituting 9-phenanthrol for 2-naphthol, the title compound was prepared as a purple solid (0.048 g, 16%): ^{1}H NMR (d_6-DMSO) δ 11.2 (br s, 1H), 9.25 (d, J = 8.6 Hz, 1H), 8.90 (d, J = 8.3 Hz, 1H), 8.60 (d, J = 8.3 Hz, 1H), 8.51 (t, J = 7.8 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 7.94 (s, 1H), 7.86 (t, j = 8.2 Hz, 1H), 7.66−7.48 (m, 5H).

3-Hydroxy-4-[(2-hydroxy-5-methyl-1-phenyl)azo]-1-naphthalenesulfonic acid (5) was purchased from Aldrich Chemical Co., Milwaukee, WI, and used as provided.

4-[(2-Hydroxy-1-naphthalenyl)azo]-1-naphthalene-sulfonic acid (8a) was purchased from Aldrich Chemical Co., Milwaukee, WI, and used as provided.

2,2′-Dihydroxy-1,1′-azonaphthalene (8b) was prepared according to a literature method.[50] A solution of 2-naphthol (2.88 g, 0.02 mol) in anhydrous diethyl ether (30.0 mL) was treated portionwise with sodium metal (0.23 g, 0.01 mol) and stirred and heated under reflux until all sodium had reacted. A solution of *p*-toluenesulfonyl azide (2.0 g, 0.01 mol) in anhydrous diethyl ether (20.0 mL) was then added dropwise, and the solution was stirred at room temperature overnight. The solution was filtered, and the purple solid was dissolved in 50% aqueous acetone (50.0 mL) and acidified with concentrated hydrochloric acid. Filtration afforded the title compound (0.73 mg, 23%) as a red-brown solid.

2,2′-Dihydroxy-4-nitro-1,1′-azonaphthalene (8c). (a) 1-Diazo-2-hydroxy-4-nitronaphthalene was prepared according to a literature method.[51] A suspension of *N*-(2,4-dinitronaphthalen-1-yl)acetamide (19.8 g, 0.068 mol) in water (30.0 mL) was treated with concentrated sulfuric acid (200 mL), resulting in an exothermic reaction to 80 °C. After 5 min, the mixture was cooled to 5 °C, and a solution of sodium nitrite (6.6 g, 0.096 mol) in concentrated sulfuric acid (20.0 mL) was added dropwise. After being stirred for 15 min at 10 °C, the mixture was poured into water (1 L) and filtered to afford the title compound (11.6 g, 80%) as a yellow solid.

(b) 2,2′-Dihydroxy-4-nitro-1,1′-azonaphthalene (8c). Following the procedure of **2a**, except substituting 1-diazo-2-hydroxy-4-nitronaphthalene for 1-diazo-2-naphthol-4-sulfonic acid, the title compound was prepared as a dark-brown solid (2.6 g, 72%): ^{1}H NMR (d_6-DMSO) δ 9.6 (br s, 1H), 7.85 (s, 1H), 7.82−7.73 (m, 3H), 7.67 (d, J = 7.5 Hz, 1H), 7.61 (td, J = 7.5 and 2.2 Hz, 1H), 7.39−7.24 (m, 5H).

3-Hydroxy-4-{[(2-hydroxynaphthalen-1-yl)methylene]-amino}naphthalene-1-sulfonic Acid, Triethylammonium Salt (10a). A solution of 4-amino-3-hydroxynaphthalene-1-sulfonic acid (2.4 g, 0.01 mol), 2-hydroxynaphthalene-1-carbaldehyde (1.7 g, 0.01 mol), and triethylamine (2.0 mL, 0.02 mol) in ethanol (10.0 mL) was stirred and heated under reflux for 1 h. Upon cooling, the product crystallized and was collected as an orange solid (2.43 g, 49%): ^{1}H NMR (d_6-DMSO) δ 9.96 (d, J = 4.0 Hz, 1H), 8.82 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.03−7.96 (m, 3H), 7.86 (d, J = 8.0 Hz, 1H), 7.59−7.53 (m, 2H), 7.44−7.35 (m, 2H), 7.14 (d, J = 9.1 Hz, 1H), 3.10 (q, J = 7.3 Hz, 6H), 1.17 (t, J = 7.3. Hz, 9H).

3-Hydroxy-4-{[(2-hydroxynaphthalen-1-yl)methyl]-amino}naphthalene-1-sulfonic Acid (10b). A solution of 3-hydroxy-4-{[(2-hydroxynaphthalen-2-yl)methylene]amino}-naphthalene-1-sulfonic acid, triethylammonium salt (**10a**) (0.247 g, 0.5 mmol) in anhydrous THF (5.0 mL) at −78 °C was treated dropwise with borane−THF complex (1.0 M solution in THF; 2.5 mL, 2.5 mmol) and left to warm to room temperature over 24 h. Methanol (5.0 mL) and 3 M aqueous HCl (5.0 mL) were then added, and the solution was evaporated to remove THF. Filtration and washing with water and then ethanol afforded the title compound as a cream solid (0.176 g, 89%): ^{1}H NMR (d_6-DMSO) δ 11.5 (br s, 1H), 10.6 (br s, 3H), 8.85 (d, J = 8.6 Hz, 1H), 8.21 (d, J = 8.6 Hz, 1H), 8.15 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 7.89 (m, 2H), 7.63−7.56 (m, 2H), 7.48−7.38 (m, 2H), 7.30 (d, J = 8.9 Hz, 1H), 4.90 (s, 2H).

3-Hydroxy-4-{[1-(2-hydroxynaphthalen-1-yl)methanoyl]-amino}naphthalene-1-sulfonic Acid (10c). (a) 2-(Benzyloxy)naphthalene-1-carbaldehyde (12a) was prepared according to a literature procedure.[51,53] A mixture of 2-hydroxynaphthalene-1-carbaldehyde (**11**) (20.0 g, 0.116 mol), benzyl chloride (19.1 g, 0.151 mol), and potassium carbonate (16.0 g, 0.116 mol) in ethanol (200 mL) was stirred and heated under reflux for 2 d. Water (200 mL) was added, and the mixture was extracted with dichloromethane (3×). The extracts were dried and evaporated, and the residue was crystallized from hexanes−ethyl acetate to afford the title compound as colorless needles (13.6 g, 45%): mp 120−122 °C (lit.[51,53] mp 123−124 °C).

(b) 2-(Benzyloxy)naphthalene-1-carboxylic acid (12b) was prepared according to a literature procedure.[51,53] A solution of aldehyde **12a** (49.0 g, 0.18 mol) and sulfamic acid (38.4 g, 0.40 mol.) in acetone (920 mL) and water (460 mL) was cooled to 5 °C and treated portionwise with sodium chlorite (23.4 g, 0.21 mol) over 20 min. The mixture was then stirred at 5 °C for 1 h. The mixture was evaporated to remove acetone and then extracted with dichloromethane (3×). The extracts were dried and evaporated, and the residue was triturated with hexanes to give the title compound as a cream solid (47.2 g, 91%): mp 118−120 °C. (lit.[51,53] mp 127−128 °C).

(c) 2-(Benzyloxy)naphthalene-1-carbonyl chloride (12c) was prepared according to a literature procedure.[51,53] Oxalyl chloride (25.9 mL, 137 mmol) was added to a mixture of acid **12b** (19.12 g, 68.7 mmol) and DMF (4.34 mL) in dry toluene (325 mL), and the solution was stirred at room temperature for 1 h. The toluene phase was separated from the DMF phase and concentrated. The residue was azeotroped with toluene (200 mL) to afford the title compound **12c** as a cream solid (19.6 g, 96%): mp 88−90 °C.

(d) 3-Hydroxy-4-{{1-[2-(benzyloxy)naphthalen-1-yl]-methanoyl}amino}naphthalene-1-sulfonic Acid (10c). A solution of the amine **9** (1.8 g, 7.5 mmol) and acid chloride **12c** (1.5 g, 5 mmol) in pyridine (25.0 mL) was treated with DMAP (20 mg), and the mixture was stirred at room temperature for 24 h. The mixture was partitioned between 3 M aqueous HCl (200 mL) and ethyl acetate (200 mL). The layers were separated and further extracted with ethyl acetate (2×). The organic layers were washed with further 3 M aqueous HCl (3×), dried, and evaporated, and the residue was purified by chromatography [silica gel, gradient elution, dichloromethane/ethyl acetate/acetic acid (90:10:0.1 to 80:20:0.1)] to afford the amide **12d** as an orange foam (114 mg, 4%): ^{1}H NMR (d_6-DMSO) δ 11.01 (s, 1H), 8.73 (d, J = 7.4 Hz, 1H), 8.33 (d, J = 7.7 Hz, 1H), 8.07−7.90 (m, 4H), 7.71−7.25 (m, 9H), 6.97 (s, 1H), 5.44 (s, 1H).

(e) 3-Hydroxy-4-{[1-(2-hydroxynaphthalen-1-yl)methanoyl]amino}naphthalene-1-sulfonic Acid (10c). A solution of the benzyl ether **12d** (74.5 mg; 0.15 mmol.) in methanol

(25.0 mL) was hydrogenated over 10% Pd−C (75 mg) at room temperature and 50 psi for 3 h. The mixture was filtered and evaporated and the residue triturated with ethyl acetate to afford the amide **10c** as a tan powder (32.7 mg, 53%): ^{1}H NMR (d_6-DMSO) δ 11.1−9.9 (br s, 2H), 8.77 (d, J = 8.5 Hz, 1H), 8.6 (br s, 1H), 8.33 (d, 8.5 Hz, 1H), 7.79−7.75 (m, 3H), 7.49−7.42 (m, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.24 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 8.9 Hz, 1H).

General Method for the Synthesis of 1,3-Substituted 2,4-Dihydropyrazol-3-ones (15). 5-Methyl-2-pyridin-2-yl-2,4-dihydropyrazol-3-one (15a). A solution of 2-hydrazinopyridine (1.35 g, 0.012 mol) and ethyl acetoacetate (1.60 mL, 0.012 mol) in glacial acetic acid (50.0 mL) was stirred and heated at 100 °C for 24 h. The solvent was evaporated and the product purified by chromatography (silica gel, 50% ethyl acetate/hexanes) to afford the title compound (0.78 g, 37%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 8.23 (d, J = 3.6 Hz, 1H), 7.85 (d, J = 3.6 Hz, 2H), 7.11 (m, 1H), 5.43 (br, s, 1H), 2.26 (s, 3H).

2-Benzyl-5-methyl-2,4-dihydropyrazol-3-one (15b). Following the procedure of **15a**, except substituting benzylhydrazine for 2-hydrazinopyridine, the title compound was prepared (0.74 g, 77%): ^{1}H NMR (d_6-DMSO) δ 7.39−7.18 (m, 6H), 5.49 (s, 1H), 5.08 (s, 2H), 2.13 (s, 3H).

2-(2-Hydroxyethyl)-5-methyl-2,4-dihydropyrazol-3-one (15c). Following the procedure of **15a**, except substituting 2-hydrazinoethanol for 2-hydrazinopyridine, the title compound was prepared (1.0 g, 14%): ^{1}H NMR (d_6-DMSO) δ 5.46 (s, 1H), 3.91 (m, 2H), 3.65 (m, 2H), 2.14 (s, 3H).

5-Methyl-2-(2-nitrophenyl)-2,4-dihydropyrazol-3-one (15d). Following the procedure of **15a**, except substituting 2-nitrophenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (1.8 g, 47%): ^{1}H NMR ($CDCl_3$) δ 8.07 (d, J = 6.8 Hz, 1H), 7.92 (d, J = 6.9 z, 1H), 7.83−7.39 (m, 3H), 5.85 (s, 1H), 2.40 (s, 3H).

5-Methyl-2-(3-nitrophenyl)-2,4-dihydropyrazol-3-one (15e). Following the procedure of **15a**, except substituting 3-nitrophenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (3.2 g, 89%): ^{1}H NMR ($CDCl_3$) δ 8.75 (d, J = 1.2 Hz, 1H), 8.33 (dd, J = 8.2 and 1.2 Hz, 1H), 8.03 (dd, J = 8.5 and 1.2 Hz, 1H), 7.56 (dd, J = 8.5 and 8.2 Hz, 1H), 3.50 (s, 2H), 2.25 (s, 3H).

5-Methyl-2-(2-methylphenyl)-2,4-dihydropyrazol-3-one (15f). Following the procedure of **15a**, except substituting 2-methylphenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (3.2 g, 68%): ^{1}H NMR ($CDCl_3$) δ 7.35−7.32 (m, 4H), 3.42 (s, 2H), 2.29 (s, 3H), 2.15 (s, 3H).

5-Methyl-2-(3-methylphenyl)-2,4-dihydropyrazol-3-one (15g). Following the procedure of **15a**, except substituting 3-methylphenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (2.3 g, 90%): ^{1}H NMR ($CDCl_3$) δ 8.68 (m, 2H), 7.25 (m, 1H), 7.00 (d, J = 2.3 Hz, 1H), 3.43 (s, 2H), 2.38 (s, 3H), 2.18 (s, 3H).

5-Methyl-2-[3-(trifluoromethyl)phenyl]-2,4-dihydropyrazol-3-one (15h). Following the procedure of **15a**, except substituting 3-(trifluoromethyl)phenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (0.78 g, 76%): ^{1}H NMR ($CDCl_3$) δ 8.19−8.15 (m, 2H), 7.52 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 3.48 (s, 2H), 2.32 (s, 3H).

5-Methyl-2-[4-(trifluoromethyl)phenyl]-2,4-dihydropyrazol-3-one (15i). Following the procedure of **15a**, except substituting 4-(trifluoromethyl)phenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (3.25 g, 93%): ^{1}H NMR ($CDCl_3$) δ 8.07 (d, J = 7.8 Hz, 2H), 7.65 (d, J = 7.8 Hz, 2H), 3.48 (s, 2H), 2.23 (s, 3H).

2-(4-Iodophenyl)-5-methyl-2,4-dihydropyrazol-3-one (15j). Following the procedure of **15a**, except substituting 4-iodophenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (2.20 g, 75%): ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 1H), 7.69−7.65 (m, 3H), 3.44 (s, 2H), 2.21 (s, 3H).

2-[4-(Benzyloxy)phenyl]-5-methyl-2,4-dihydropyrazol-3-one (15k). (a) 4-(Benzyloxy)phenylhydrazine. A solution of 4-(benzyloxy)aniline hydrochloride (11.3 g, 0.048 mol) in concentrated hydrochloric acid (40.0 mL) was cooled to 0 °C and then treated dropwise with a solution of sodium nitrite (3.28 g, 0.048 mol) in water (20.0 mL). The mixture was stirred at 0 °C for a further 10 min and then poured into a cold (−10 °C) solution of tin dichloride hydrate (40.0 g, 0.18 mol) in concentrated hydrochloric acid (40.0 mL). The mixture was allowed to warm to room temperature with stirring for 1 h. The mixture was basified with 10% aqueous sodium hydroxide, ethyl acetate (1 L) was added, and the mixture was filtered to remove unwanted tin residues. The organic layer was then dried and evaporated to afford the title compound as a yellow solid (6.9 g, 67%): mp 105−107 °C.

(b) 2-[4-(Benzyloxy)phenyl]-5-methyl-2,4-dihydropyrazol-3-one (15k). Following the procedure of **15a**, except substituting 4-(benzyloxy)phenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (1.57 g, 59%): ^{1}H NMR ($CDCl_3$) δ 11.3 (br s, 1H), 7.57 (d, J = 9.1 Hz, 2H), 7.54−7.32 (m, 5H), 7.07 (d, J = 9.2 Hz, 2H), 5.32 (s, 1H), 5.13 (s, 2H), 2.10 (s, 3H).

2-(3,4-Dichlorophenyl)-5-methyl-2,4-dihydropyrazol-3-one (15l). Following the procedure of **15a**, except substituting 3,4-dichlorophenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (5.0 g, 77%): ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.9 and 2.4 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 5.3 (br s, 1H), 2.12 (s, 3H).

2-(3,4-Dimethylphenyl)-5-methyl-2,4-dihydropyrazol-3-one (15m). Following the procedure of **15a**, except substituting 3,4-dimethylphenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (16.8 g, 64%): ^{1}H NMR ($CDCl_3$) δ 7.65 (d, J = 1.8 Hz, 1H), 7.54 (dd, J = 8.6 and 1.8 Hz, 1H), 7.13 (d, J = 8.6 Hz, 1H), 3.40 (s, 2H), 2.31 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H).

5-Methyl-2-[4-(2-methylethyl)phenyl]-2,4-dihydropyrazol-3-one (15n). Following the procedure of **15a**, except substituting 4-(2-methylethyl)phenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (2.1 g, 60%): ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 3.40 (s, 2H), 2.89 (m, 1H), 2.11 (s, 3H), 1.22 (d, J = 7.3 Hz, 6H).

2-(3-*tert*-Butylphenyl)-5-methyl-2,4-dihydropyrazol-3-one (15o). Following the procedure of **15a**, except substituting 3-*tert*-butylphenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (5.1 g, 75%): ^{1}H NMR ($CDCl_3$) δ 7.85 (td, J = 2.0 and 1.9 Hz, 1H), 7.66 (ddd, J = 8.6, 2.3 and 1.9 Hz, 1H), 7.32 (t, J = 8.6 Hz, 1H), 7.22 (ddd, J = 8.6 Hz, 2.0 and 1.9 Hz, 1H), 3.42 (s, 2H), 2.21 (s, 3H), 1.31 (s, 9H).

2-(4-*tert*-Butylphenyl)-5-methyl-2,4-dihydropyrazol-3-one (15p). Following the procedure of **15a**, except substituting 4-*tert*-butylphenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (13.8 g, 60%): ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 9.3 Hz, 2H), 7.41 (d, J = 9.3 Hz, 2H),3.43 (s, 2H), 2.20 (s, 3H), 1.32 (s, 9H).

2-[4-(Methylsulfonyl)phenyl]-5-methyl-2,4-dihydropyrazol-3-one (15q). Following the procedure of **15a**, except substituting 4-(methylsulfonyl)phenylhydrazine for 2-hydrazinopyridine, the title compound was prepared (0.95 g; 65%): ^{1}H NMR (d_6-DMSO) δ 12.0 (br s, 1H), 8.10−7.90 (m, 5H), 5.40 (s, 1H), 3.21 (s, 3H), 2.13 (s, 3H).

5-*tert*-Butyl-2-(3,4-dimethylphenyl)-2,4-dihydropyrazol-3-one (15r). Following the procedure of **15a**, except substituting 3,4-dimethylphenylhydrazine for 2-hydrazinopyridine and ethyl 4,4-dimethyl-3-oxopentanoate for ethyl acetoacetate, the title compound was prepared (2.3 g, 55%): ^{1}H NMR ($CDCl_3$) δ 7.64 (d, J = 2.1 Hz, 1H), 7.57 (dd, J = 8.2 and 2.1 Hz, 1H), 7.13 (d, J = 8.2 Hz, 1H), 3.44 (s, 2H), 2.29 (s, 3H), 2.25 (s, 3H), 1.27 (s, 9H).

2-(3,4-Dimethylphenyl)-5-phenyl-2,4-dihydropyrazol-3-one (15s). Following the procedure of **15a**, except substituting 3,4-dimethylphenylhydrazine for 2-hydrazinopyridine and ethyl benzoylacetate for ethyl acetoacetate, the title compound was prepared (5.5 g, 80%): ^{1}H NMR (d_6-DMSO) δ 11.7 (br s, 1H), 7.82−7.79 (m, 2H), 7.57 (s, 1H), 7.49 (dd, J = 8.2 and 2.0 Hz, 1H), 7.40 (t, J = 7.7 Hz, 1H), 7.31 (m, 1H), 7.22 (d, J = 8.2 Hz, 1H), 5.99 (s, 1H), 2.28 (s, 3H), 2.25 (s, 3H).

1-(4-*tert*-Butylphenyl)-5-oxo-4,5-dihydropyrazole-3-carboxylic Acid Ethyl Ester (15t). Following the procedure of **15a**, except substituting 4-*tert*-butylphenylhydrazine for 2-hydrazinopyridine and 2-oxosuccinic acid diethyl ester for ethyl acetoacetate, the title compound was prepared (3.0 g, 70%) as a colorless solid. ^{1}H NMR ($CDCl_3$) showed a 1:1 mixture of keto/enol tautomers. Anal. ($C_{16}H_{20}N_2O_3$) Calcd: C, 66.7; H, 7.0; N, 9.7. Found: C, 66.7; H, 7.2; N, 9.9.

1-(4-*tert*-Butylphenyl)-5-oxo-4,5-dihydropyrazole-3-carboxylic Acid (15u). Following the procedure of **15a**, except substituting 4-*tert*-butylphenylhydrazine for 2-hydrazinopyridine and oxalacetic acid for ethyl acetoacetate, the title compound was prepared (0.25 g, 96%): ^{1}H NMR ($CDCl_3$) δ 7.23 (d, J = 8.1 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H), 3.28 (s, 1H), 1.22 (s, 9H).

1-(4-*tert*-Butylphenyl)-5-oxo-4,5-dihydropyrazole-3-carboxylic Acid tert-Butyl Ester (15v). A suspension of pyrazolecarboxylic acid **15u** (5.0 g, 0.019 mol) and concentrated sulfuric acid (1.5 mL) in dichloromethane (80.0 mL) at −70 °C in a pressure bottle was saturated with isobutylene (∼10 mL), sealed, and then allowed to warm to room temperature for 24 h. The mixture was then washed with water and sodium hydrogen carbonate, dried, and evaporated and the residue triturated with diethyl ether to afford the title compound (0.94 g, 16%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 3.65 (s, 2H), 1.63 (s, 9H), 1.28 (s, 9H).

4-[*N*′-(3-Methyl-5-oxo-1,5-dihydropyrazol-4-ylidene)hydrazino]-3-hydroxynaphthalene-1-sulfonic Acid (24a). Following the procedure of **2a**, except substituting 5-methyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared (1.21 g, 82%) as a red solid: ^{1}H NMR (d_6-DMSO) δ 11.1 (br s, 1H), 8.87−8.82 (m, 2H), 7.83 (s, 1H), 7.53 (dd, J = 8.4 and 6.9 Hz, 1H), 7.40 (dd, J = 8.5 and 6.9 Hz, 1H), 2.25 (s, 3H).

4-[*N*′-(1,3-Dimethyl-5-oxo-1,5-dihydropyrazol-4-ylidene)-hydrazino]-3-hydroxynaphthalene-1-sulfonic Acid, Monohydrate, Monosodium Salt (24b). Following the procedure of **2a**, except substituting 2,5-dimethyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared (1.95 g, 49%) as a red solid: ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.0 (br s, 1H), 8.86 (t, J = 8.9 Hz, 2H), 7.86 (s, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.40 (t, J = 7.5 Hz, 1H), 3.32 (s, 3H), 2.24 (s, 3H). Anal. ($C_{15}H_{14}N_4OS\cdot Na\cdot H_2O$) Calcd: C, 44.8; H, 4.0; N, 13.9. Found: C, 44.6; H, 3.7; N, 13.7.

4-[*N*′-(1-*tert*-Butyl-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene)hydrazino]-3-hydroxynaphthalene-1-sulfonic Acid (24c). Following the procedure of **2a**, except substituting 2-*tert*-butyl-5-methyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (0.0663 g, 24%): ^{1}H NMR (d_6-DMSO) δ 14.2 (br s, 1H), 11.0 (br s, 1H), 8.88−8.82 (m, 2H), 7.85 (s, 1H), 7.52 (m, 1H), 7.38 (m, 1H), 2.21 (s, 3H), 1.52 (s, 9H).

3-Hydroxy-4-[*N*′-(3-methyl-5-oxo-1-phenyl-1,5-dihydropyrazol-4-ylidene)hydrazino]naphthalene-1-sulfonic Acid, Monosodium Salt (24d). Following the procedure of **2a**, except substituting 5-methyl-2-phenyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (3.82 g, 90%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.4 Hz, 1H), 8.87 (d, J = 8.5 Hz, 1H), 7.98 (d, J = 8.1 Hz, 2H), 7.89 (s, 1H), 7.58 (t, J = 8.3 Hz, 1H), 7.50−7.40 (m, 3H), 7.22 (t, J = 7.3 Hz, 1H), 2.39 (s, 3H).

3-Hydroxy-4-[*N*′-(3-methyl-5-oxo-1-pyridin-2-yl-1,5-dihydropyrazol-4-ylidene)hydrazino]-naphthalene-1-sulfonic Acid (24e). Following the procedure of **2a**, except substituting compound **15a** for 2-naphthol, the title compound was prepared as a red solid (1.33 g, 73%): ^{1}H NMR (d_6-DMSO) δ 11.2 (br s, 1H), 8.97 (d, J = 8.6 Hz, 1H), 8.87 (d, J = 8.2 Hz, 1H), 8.50 (s, 1H), 8.00 (d, J = 2.1 Hz, 1H), 7.89 (s, 2H), 7.61−7.34 (m, 3H), 2.40 (s, 3H).

4-[*N*′-(1-Benzyl-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene)hydrazino]-3 -hydroxynaphthalene-1-sulfonic Acid (24f). Following the procedure of **2a**, except substituting compound **15b** for 2-naphthol, the title compound was prepared as a red solid (0.07 g, 30%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.0 (br s, 1H), 8.91−8.82 (m, 2H), 7.85 (s, 1H), 7.54 (m, 1H), 7.42−7.29 (m, 6H), 4.9 (s, 2H), 2.23 (s, 3H).

3-Hydroxy-4-{*N*′-[1-(2-hydroxyethyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (24g). Following the procedure of **2a**, except substituting compound **15c** for 2-naphthol, the title compound was prepared as a red solid (0.10 g, 55%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.1 (br s, 1H), 8.84 (m, 2H), 7.84 (s, 1H), 7.52 (m, 1H), 7.39 (m, 1H), 3.72 (m, 2H), 3.65 (m, 2H), 2.23 (s, 3H).

4-{*N*′-[1-(2-Chlorophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25a). Following the procedure of **2a**, except substituting 2-(2-chlorophenyl)-5-methyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (2.7 g, 99%): ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.1 (br s, 1H), 8.95 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.5 z, 1H), 7.87 (s, 1H), 7.70−7.50 (m, 5H), 7.43 (t, J = 7.6 Hz, 1H), 2.35 (s, 3H).

4-{*N*′-[1-(3-Chlorophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25b). Following the procedure of **2a**, except substituting 2-(3-chlorophenyl)-5-methyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (0.69 g, 50%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.2 (br s, 1H), 8.97 (d, J = 8.6 Hz, 1H), 8.87 (d, J = 8.3 Hz, 1H), 8.06 (d, J = 1.7 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.91 (s, 1H), 7.84−7.28 (m, 3H), 7.16 (d, J = 8.4 Hz, 1H), 2.39 (s, 3H).

4-{*N*′-[1-(4-Chlorophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25c). Following the procedure of **2a**, except substituting 2-(4-chlorophenyl)-5-methyl-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (1.8 g, 97%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.2 (br s, 1H), 8.97 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.9 Hz, 2H), 7.89 (s, 1H), 7.61−7.52 (m, 3H), 7.43 (t, J = 7.7 Hz, 1H), 2.38 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(2-nitrophenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25d). Following the procedure of **2a**, except substituting pyrazole **15d** for 2-naphthol, the title compound was prepared as a red solid (0.067 g, 5%): ^{1}H NMR (d_6-DMSO) δ 13.8 (br s, 1H), 11.2 (br s, 1H), 8.92 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.87 (s, 1H), 7.85−7.80 (m, 2H), 7.63−7.56 (m, 2H), 7.43 (t, J = 7.9 Hz, 1H), 2.35 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(3-nitrophenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic acid (25e). Following the procedure of **2a**, except substituting compound **15e** for 2-naphthol, the title compound was prepared as a red solid (2.4 g, 92%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.3 (br s, 1H), 8.98 (d, J = 8.5 Hz, 1H), 8.89−8.73 (m, 2H), 8.43 (dd, J = 8.1 and 1.2 Hz, 1H), 8.07 (dd, J = 8.1 and 1.8 Hz, 1H), 7.90 (s, 1H), 7.78 (t, J = 8.2 Hz, 1H), 7.60 (t, J = 8.1 Hz, 1H), 7.44 (t, J = 7.7 Hz, 1H), 2.42 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(4-nitrophenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25f). Following the procedure of **2a**, except substituting 5-methyl-2-(4-nitrophenyl)-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (2.0 g, 99%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.3 (br s, 1H), 8.99 (d, J = 8.9 Hz, 1H), 8.88 (d, J = 8.6 Hz, 1H), 8.38 (d, J = 9.3 Hz, 2H), 8.28 (d, J = 9.3 Hz, 2H), 7.90 (s, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 2.42 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(2-methylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25g). Following the procedure of **2a**, except substituting pyrazole **15f** for 2-naphthol, the title compound was prepared as a red solid (0.092 g, 38%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.0 (br s, 1H), 8.97 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 7.90 (s, 1H), 7.53 (m, 1H), 7.40−7.20 (m, 5H), 2.38 (s, 3H), 2.30 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(3-methylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25h). Following the procedure of **2a**, except substituting pyrazole **15g** for 2-naphthol, the title compound was prepared as a red solid (1.18 g, 48%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 7.89 (s, 1H), 7.81−7.77 (m, 2H), 7.58 (dd, J = 8.2 and 7.0 Hz, 1H), 7.43 (t, J = 8.2 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 2.38 (s, 6H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(4-methylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25i). Following the procedure of **2a**, except substituting 5-methyl-2-(4-methylphenyl)-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (2.1 g, 81%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.6 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 7.90 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.58 (td, J = 8.2 and 1.0 Hz, 1H), 7.43 (t, J = 7.4 Hz, 1H), 7.28 (d, J = 8.5 Hz, 1H), 2.37 (s, 3H), 2.34 (s, 3H).

3-Hydroxy-4-{*N*′-{3-methyl-5-oxo-1-[3-(trifluoromethyl)phenyl]-1,5-dihydropyrazol-4-ylidene}hydrazino}-naphthalene-1-sulfonic Acid (25j). Following the procedure of **2a**, except substituting pyrazole **15h** for 2-naphthol, the title compound was prepared as a red solid (1.19 g, 72%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.3 (br s, 1H), 8.98 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 8.34 (s, 1H), 8.29 (d, J = 8.3 Hz, 1H), 7.90 (s, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.60 (m, 1H), 7.44 (t, J = 8.2 Hz, 1H), 2.40 (s, 3H).

3-Hydroxy-4-{*N*′-{3-methyl-5-oxo-1-[4-(trifluoromethyl)phenyl]-1,5-dihydropyrazol-4-ylidene}hydrazino}-naphthalene-1-sulfonic Acid (25k). Following the procedure of **2a**, except substituting pyrazole **15i** for 2-naphthol, the title compound was prepared as a red solid (1.64 g, 77%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.98 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 8.23 (d, J = 8.5 Hz, 2H), 7.91 (s, 1H), 7.84 (d, J = 8.5 Hz, 2H), 7.59 (t, J = 8.0 Hz, 1H), 7.42 (t, J = 8.2 Hz, 1H), 2.40 (s, 3H).

4-{*N*′-[1-(4-Fluorophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25l). Following the procedure of **2a**, except substituting 5-methyl-2-[4-(fluoromethyl)phenyl]-2,4-dihydropyrazol-3-one for 2-naphthol, the title compound was prepared as a red solid (2.03 g, 75%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.5 Hz, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.01−7.96 (m, 2H), 7.89 (s, 1H), 7.58 (td, J = 8.2 and 1.2 Hz, 1H), 7.35−7.30 (m, 2H), 2.38 (s, 3H).

3-Hydroxy-4-{*N*′-[1-(4-iodophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25m). Following the procedure of **2a**, except substituting pyrazole **15j** for 2-naphthol, the title compound was prepared as a red solid (0.50 g, 45%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.97 (d, J = 8.6 Hz, 1H), 8.86 (d, J = 8.6 Hz, 1H), 7.85 (s, 1H), 7.82 (m, 4H), 7.58 (m, 1H), 7.42 (m, 1H), 2.38 (s, 3H).

4-{*N*′-{1-[4-(Benzyloxy)phenyl]-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene}hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25n). Following the procedure of **2a**, except substituting pyrazole **15k** for 2-naphthol, the title compound was prepared as a red solid (0.50 g, 45%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.96 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.7 Hz, 1H), 7.89 (s, 1H), 7.86 (d, J = 8.9 Hz, 2H), 7.60−7.3 (m, 6H), 7.13 (d, J = 8.9 Hz, 1H), 5.15 (s, 2H), 2.37 (s, 3H).

4-{*N*′-[1-(3,4-Dichlorophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25o). Following the procedure of **2a**, except substituting pyrazole **15l** for 2-naphthol, the title compound was prepared as a red solid (1.89 g, 77%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.3 (br s, 1H), 8.96 (d, J = 8.7 Hz, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.19 (d, J = 2.3 Hz, 1H), 7.94 (dd, J = 8.9 and 2.3 Hz, 1H), 7.93 (s, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.57 (dd, J = 8.8 and 8.7 Hz, 1H), 7.42 (dd, J = 8,8 and 8.5 Hz, 1H), 2.34 (s, 3H).

3-Hydroxy-4-{*N*′-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25p). Following the procedure of **2a**, except substituting pyrazole **15m** for 2-naphthol, the title compound was prepared as a red solid (23.3 g, 97%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.4 Hz, 1H), 8.86 (d, J = 8.4 Hz, 1H), 7.89 (s, 1H), 7.76 (s, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.57 (t, J = 8.4 Hz, 1H), 7.42 (t, J = 8.4 Hz, 1H), 7.21 (d, J = 8.1 Hz, 1H), 2.37 (s, 3H), 2.29 (s, 3H), 2.24 (s, 3H).

3-Hydroxy-4-{*N*′-{3-methyl-1-[4-(2-methylethyl)phenyl]-5-oxo-1,5-dihydropyrazol-4-ylidene}hydrazino}naphthalene-1-sulfonic Acid (25q). Following the procedure of **2a**, except substituting pyrazole **15n** for 2-naphthol, the title compound was prepared as a red solid (0.20 g, 90%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.8 Hz, 1H), 8.87 (d, J = 8.6 Hz, 1H), 7.91 (s, 1H), 7.82 (d, J = 8.5 Hz, 2H), 7.49 (m, 1H), 7.37 (m, 1H), 7.28 (d, J = 8.5 Hz, 2H), 2.92 (m, 1H), 2.34 (s, 3H), 1.25 (d, J = 7.3 Hz, 6H).

4-{*N*′-[1-(3-*tert*-Butylphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25r). Following the procedure of **2a**, except substituting pyrazole **15o** for 2-naphthol, the title compound was prepared as a red solid (0.14 g, 64%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.97 (d, J = 8.4 Hz, 1H), 8.86 (d, J = 8.4 Hz, 1H), 8.03 (t, J = 2.3 Hz, 1H), 7.89 (s, 1H), 7.79 (m, 1H), 7.58 (m, 1H), 7.43−7.38 (m, 2H), 7.28 (m, 1H), 2.38 (s, 3H), 1.18 (s, 9H).

4-{*N*′-[1-(4-*tert*-Butylphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25s). Following the procedure of **2a**, except substituting pyrazole **15p** for 2-naphthol, the title compound was prepared as a red solid (5.4 g, 98%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.97 (d, J = 8.8 Hz, 1H), 8.86 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 7.88 (d, J = 8.6 Hz, 2H), 7.57 (m, 1H), 7.49 (d, J = 8.6 Hz, 2H), 7.43 (m, 1H), 2.37 (s, 3H), 1.31 (s, 9H).

3-Hydroxy-4-{*N*′-{1-[4-(methylsulfonyl)phenyl]-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene}hydrazino}naphthalene-1-sulfonic Acid (25t). Following the procedure of **2a**, except substituting pyrazole **15q** for 2-naphthol, the title compound was prepared as a red solid (0.21 g, 35%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.1 (br s, 1H), 8.93 (d, J = 8.6 Hz, 1H), 8.82 (d, J = 8.6 Hz, 1H), 8.21 (d, J = 8.9 Hz, 2H), 7.97 (d, J = 8.9 Hz, 1H), 7.85 (s, 1H), 7.54 (m, 1H), 7.39 (m, 1H), 3.19 (s, 3H), 2.35 (s, 3H).

4-{*N*′-{1-[3-(Ethylsulfamoyl)phenyl]-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene}hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25u). Following the procedure of **2a**, except substituting *N*-ethyl-3-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzenesulfonamide for 2-naphthol, the title compound was prepared as a red solid (1.92 g, 86%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.99 (d, J = 8.6 Hz, 1H), 8.88 (d, J = 8.5 Hz, 1H), 8.45 (s, 1H), 8.27 (d, J = 7.2 Hz, 1H), 7.91 (s, 1H), 7.71 (m, 1H), 7.61 (m, 1H), 7.44 (m, 1H) 2.84 (m, 2H), 2.42 (s, 3H), 1.00 (t, J = 7.1 Hz, 3H).

4-{4-[(2-Hydroxy-4-sulfonaphthalen-1-yl)hydrazono]-3-methyl-5-oxo-4,5-dihydropyrazol-1-yl}benzoic Acid (25v). Following the procedure of **2a**, except substituting 4-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzoic acid for 2-naphthol, the title compound was prepared as a red solid (1.92 g, 95%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.99 (d, J = 8.7 Hz, 1H), 8.88 (d, J = 8.6 Hz, 1H), 8.14 (d, J = 7.2 Hz, 2H), 8.05 (d, J = 7.2 Hz, 2H), 7.90 (s, 1H), 7.59 (m, 1H), 7.44 (m, 1H), 2.40 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-5-oxo-1-(4-sulfophenyl)-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25w). Following the procedure of **2a**, except substituting 4-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzenesulfonic acid for 2-naphthol, the title compound was prepared as a red solid (1.82 g, 85%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.2 (br s, 1H), 8.99 (m, 1H), 8.88 (m, 1H), 7.95−7.42 (m, 7H), 2.40 (s, 3H).

4-{*N*′-[1-(2-Chloro-5-sulfophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25x). Following the procedure of **2a**, except substituting 4-chloro-3-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzenesulfonic acid for 2-naphthol, the title compound was prepared as a red solid (2.10 g, 92%): ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.1 (br s, 1H), 8.96 (d, J = 8.6 Hz, 1H), 8.86 (d, J = 8.5 Hz, 1H), 7.89 (s, 1H), 7.72−7.41 (m, 6H), 2.36 (s, 3H).

4-{*N*′-[1-(2,5-Dichloro-4-sulfophenyl)-3-methyl-5-oxo-1,5-dihydropyrazol- 4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (25y). Following the procedure of **2a**, except substituting 2,5-dichloro-4-(3-methyl-5-oxo-4,5-dihydropyrazol-1-yl)benzenesulfonic acid for 2-naphthol, the title compound was prepared as a red solid (2.00 g, 82%): ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.1 (br s, 1H), 8.94 (d, J = 8.2 Hz, 1H), 8.85 (d, J = 5.7 Hz, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.67 (s, 1H), 7.58 (m, 1H), 7.42 (m, 1H), 2.34 (s, 3H).

3-Hydroxy-4-{*N*′-[1-(4-hydroxyphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (25z). A solution of benzyloxy compound **25n** (0.42 g, 0.79 mmol) and 2,3,4,5,6-pentamethylbenzene (1.15 g; 7.9 mmol) in trifluoroacetic acid (5.0 mL) was stirred at room temperature for 48 h. The mixture was evaporated and the residue purified by chromatography [ODS silica, gradient elution, 10−90% acetonitrile/water (0.01% TFA)] to afford the title compound (0.15 g, 42%): ^{1}H NMR (d_6-DMSO) δ 14.1 (br s, 1H), 11.1 (br s, 1H), 8.96 (d, J = 8.7 Hz, 1H), 8.86 (d, J = 8.6 Hz, 1H), 7.88 (s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.57 (m, 1H), 7.42 (m, 1H), 6.86 (d, J = 8.8 Hz, 2H), 5.15 (s, 2H), 2.35 (s, 3H).

3-Hydroxy-4-{*N*′-[1-(3,4-dimethylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (26a). (a) 1-(3,4-Dimethylphenyl)-5-hydroxy-1*H*-pyrazole-4-carboxylic Acid Ethyl Ester (17). A suspension of 3,4-dimethylphenylhydrazine hydrochloride (2.0 g, 0.012 mol) and potassium carbonate (3.2 g, 0.023 mol) in anhydrous ethanol (20 mL) was treated with diethyl (ethoxymethylene)malonate (2.5 g, 0.012 mol), and the mixture was stirred and heated under reflux for 24 h. The mixture was evaporated, diluted with 3 M aqueous hydrochloric acid, then extracted with ethyl acetate, dried, and evaporated and the residue triturated with diethyl ether to afford the ester 17 (2.7 g, 89%) as a brown solid: ^{1}H NMR ($CDCl_3$) δ 8.2 (br s, 1H), 8.07 (s, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.34 (dd, J = 8.2 and 2.3 Hz, 1H), 7.18 (d, J = 8.2 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 2.23 (s, 3H), 2.18 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H).

(b) 2-(3,4-Dimethylphenyl)-2,4-dihydropyrazol-3-one (18). A suspension of ester **17** (1.3 g, 0.005 mol) in 10% aqueous sodium hydroxide (10.0 mL) and methanol (10.0 mL) was stirred and heated under reflux for 4 h. After being cooled, the mixture was acidified with 3 M aqueous hydrochloric acid and heated under reflux for a further 2 h. Extraction with ethyl acetate afforded the pyrazole **18a** (0.82 g, 87%) as a yellow solid: H NMR ($CDCl_3$) δ 7.61 (d, J = 2.0 Hz, 1H), 7.55 (dd, J = 8.2 and 2.2 Hz, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.26 (m, 1H), 7.16 (d, J = 8.2 Hz, 1H), 3.49 (s, 2H), 2.23 (s, 3H), 2.18 (s, 3H).

(c) 3-Hydroxy-4-{*N*′-[1-(3,4-dimethylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (26a). Following the procedure of **2a**, except substituting pyrazole **18** for 2-naphthol, the title compound was prepared as a red solid (8%): ^{1}H NMR (d_6-DMSO) δ 11.2 (br s, 1H), 8.94 (d, J = 8.6 Hz, 1H), 8.87 (d, J = 9.0 Hz, 1H), 8.23 (s, 1H), 7.8 (s, 1H), 7.77 (s, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.56 (t, J = 8.4 Hz, 1H), 7.43 (m,1H), 7.25 (d, J = 8.6 Hz, 1H), 2.32 (s, 3H), 2.24 (s, 3H).

4-{*N*′-[3-*tert*-Butyl-1-(3,4-dimethylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (26b). Following the procedure of **2a**, except substituting pyrazole **15r** for 2-naphthol, the title compound was prepared as a red solid (0.41 g, 88%): ^{1}H NMR (d_6-DMSO) δ 14.3 (br s, 1H), 11.1 (br s, 1H), 8.87−8.82 (m, 2H), 7.88 (s, 1H), 7.76 (s, 1H), 7.66 (dd, J = 8.1 and 2.0 Hz, 1H), 7.52 (m, 1H), 7.40 (m, 1H), 7.22 (d, 8.3 Hz, 1H), 2.28 (s, 3H), 2.24 (s, 3H), 1.47 (s, 9H).

3-Hydroxy-4-{*N*′-[1-(3,4-dimethylphenyl)-3-phenyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-sulfonic Acid (26c). Following the procedure of **2a**, except substituting pyrazole **15s** for 2-naphthol, the title compound was prepared as a red solid (0.32 g, 65%): ^{1}H NMR (d_6-DMSO) δ 14.5 (br s, 1H), 11.2 (br s, 1H), 8.98 (d, J = 8.6 Hz, 1H), 8.87 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 6.7 Hz, 2H), 7.91 (s, 1H), 7.85−7.76 (m, 2H), 7.60−7.41 (m, 5H), 7.27 (d, J = 8.3 Hz, 1H), 2.32 (s, 3H), 2.26 (s, 3H).

4-{*N*′-[1-(3,4-Dimethylphenyl)-3-ethoxy-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (26d). (a) Acetic Acid, *N*′-(3,4-Dimethylphenyl)hydrazide (19). 3,4-Dimethylphenylhydrazine hydrochloride (50.0 g, 0.29 mol) was partitioned between 10% sodium hydroxide and ethyl acetate, and the layers were separated. The aqueous layer was further extracted with ethyl acetate (2×), and the combined organic layers were evaporated to afford free-base phenylhydrazine, which was suspended in diethyl ether (300 mL) and cooled to 0 °C. A solution of acetic anhydride (59.0 mL, 0.63 mol.) in diethyl ether (200 mL) was then added dropwise over 15 min, and the mixture was stirred at 0 °C for a further 10 min and then diluted with hexanes (100 mL). The mixture was then filtered and the solid washed with hexanes−diethyl ether (1:1) (200 mL) to afford the title compound as a tan solid (34.0 g, 59%). ^{1}H NMR (d_6-DMSO) shows this product to be a 1:1 mixture of monoacetylated isomers.

(b) 1-(3,4-Dimethylphenyl)-1*H*,4*H*-pyrazoline-3,5-dione (20). A mixture of hydrazide **19** (31.0 g, 0.12 mol), malonic acid (18.4 g, 0.12 mol), and phosphorus trichloride (17.5 mL, 0.20 mol) was heated at 100 °C until gas evolution had ceased. After being cooled, the mixture was quenched with saturated aqueous sodium hydrogen carbonate and then extracted with diethyl ether (2×). The aqueous mother liquor was then acidified with concentrated hydrochloric acid to pH 6.5 and then extracted with ethyl acetate (4×) to afford compound **20** (4.0 g, 17%): ^{1}H NMR (d_6-DMSO) δ 7.30 (s, 1H), 7.12 (dd, J = 8.2 and 2.3 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 3.33 (s, 2H), 2.23 (s, 3H), 2.20 (s, 3H).

(c) 1-(3,4-Dimethylphenyl)-3-ethoxy-3-pyrazolin-5-one (21). A solution of pyrazoline-3,5-dione **20** (0.315 g, 1.5 mmol) in ethanol (15.0 mL) was treated with 3 drops of concentrated sulfuric acid and then heated under reflux with stirring and with removal of water through the use of a Dean−Stark apparatus containing 3 Å molecular sieves for 24 h. The reaction was cooled and quenched by addition of water. The mixture was then evaporated to give a solid which was purified by chromatography (silica gel, gradient 0−2% methanol/chloroform) to afford the ethoxypyrazole **21** (0.172 g, 48%) as a yellow solid: ^{1}H NMR (d_6-DMSO) δ 7.62 (d, J = 2.1 Hz, 1H), 7.53 (dd, J = 8.2 and 2.1 Hz, 1H), 7.12 (d, J = 8.2 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 3.41 (s, 2H), 2.23 (s, 3H), 2.20 (s, 3H), 1.40 (t, J = 7.2 Hz, 3H).

(d) 4-{*N*′-[1-(3,4-Dimethylphenyl)-3-ethoxy-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (26d). Following the procedure of **2a**, except substituting pyrazole **21** for 2-naphthol, the title compound was prepared as a red solid (60 mg, 26%): ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.0 (br s, 1H), 8.82 (t, J = 8.6 Hz, 1H), 7.89 (s, 1H), 7.75 (d, J = 2.1 Hz, 1H), 7.64 (dd, J = 8.2 and 2.1 Hz, 1H), 7.54 (td, J = 8.6 and 1.9 Hz, 1H), 7.41 (td, J = 8.6 and 2.1 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 4.43 (q, J = 7.3 Hz, 2H), 2.29 (s, 3H), 2.21 (s, 3H), 1.42 (t, J = 7.3 Hz, 1H).

1-(4-*tert*-Butylphenyl)-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)hydrazono]-5-oxo-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (26e). Following the procedure of **2a**, except substituting pyrazole **15t** for 2-naphthol, the title compound was prepared as a red solid (0.009 mg, 2%): ^{1}H NMR (d_6-DMSO) δ 11.5 (br s, 1H), 9.39 (d, J = 6.6 Hz, 1H), 8.91 (d, J = 8.5 Hz, 1H), 7.88−7.40 (m, 7H), 4.48 (q, J = 7.2 Hz, 2H), 1.45 (t, J = 7.2 Hz, 3H), 1.34 (s, 9H).

1-(4-*tert*-Butylphenyl)-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)hydrazono]-5-oxo-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid *t*-Butyl Ester (26f). Following the procedure of **2a**, except substituting pyrazole **15v** for 2-naphthol, the title compound was prepared as a red solid (0.38 mg, 67%): ^{1}H NMR (d_6-DMSO) δ 11.5 (br s, 1H), 9.26 (d, J = 8.5 Hz, 1H), 8.89 (d, J = 8.3 Hz, 1H), 7.91 (s, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.56−7.45 (m, 4H), 1.65 (s, 9H), 1.34 (s, 9H).

1-(4-*tert*-Butylphenyl)-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)hydrazono]-5-oxo-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (26g). A suspension of *tert*-butyl ester **26f** (0.11 g, 0.19 mmol) in 1,4-dioxane (5.0 mL) was treated with hydrochloric acid (4 M solution in 1,4-dioxane, 5.0 mL) and stirred at room temperature for 24 h. The mixture was evaporated and triturated with ethanol to afford the title compound (0.05 g, 51%) as a red solid: ^{1}H NMR (d_6-DMSO) δ 11.4 (br s, 1H), 9.28 (d, J = 8.0 Hz, 1H), 8.87 (d, J = 8.3 Hz, 1H), 7.92 (s, 1H), 7.88 (d, J = 8.5 Hz, 2H), 7.60−7.47 (m, 4H), 1.32 (s, 9H).

4-{*N*′-[3-Amino-1-(4-*tert*-butylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (26h).

(a) 5-Amino-2-(4-*tert*-butylphenyl)-2,4-dihydropyrazol-3-one (23). A solution of sodium (6.9 g, 0.3 mol) in ethanol (100 mL) was treated with hydrazine hydrochloride **22** (20.0 g, 0.1 mol) and ethyl cyanoacetate (11.3 g, 0.1 mol) and the mixture stirred and heated under reflux for 24 h. The mixture was evaporated, dissolved in water, and then extracted with diethyl ether. The aqueous mother liquor was neutralized with glacial acetic acid and then extracted with diethyl ether. The extracts were washed with water, dried, and evaporated, and the residue was purified by chromatography (silica, 5% methanol/dichloromethane) to afford the amine **23** (6.6 g, 29%): ^{1}H NMR ($CDCl_3$) δ 7.68 (d, J = 8.8 Hz, 2H), 7.39−7.33 (m, 2H), 6.2−5.5 (br s, 2H), 3.46 (s, 2H), 1.29 (s, 9H).

(b) 4-{*N*′-[3-Amino-1-(4-tert-butylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-sulfonic Acid (26h). Following the procedure of **2a**, except substituting pyrazole **23** for 2-naphthol, the title compound was prepared as a red solid (0.30 mg, 62%): ^{1}H NMR (d_6-DMSO) δ 13.9 (br s, 1H), 11.0 (br s, 1H), 8.87 (d, J = 8.5 Hz, 1H), 8.81 (d, J = 8.3 Hz, 1H), 7.91 (m, 2H), 7.89 (s, 1H), 7.57−7.38 (m, 4H), 1.30 (s, 9H).

3-Hydroxy-4-[*N*′-(3-methyl-5-oxo-1-phenyl-1,5-dihydropyrazol-4-ylidene)hydrazino]naphthalene-1-carboxylic Acid (30a).

(a) 3-Nitro-1-naphthalenecarboxylic Acid. A mixture of 3-nitro-1,8-naphthalic anhydride (**27**) (100 g, 0.411 mol) and sodium hydroxide (55.8 g, 1.4 mol) in water (2 L) was added to a suspension of mercury(I) oxide (96.8 g, 0.447 mol) in water (270 mL) and glacial acetic acid (200 mL). The mixture, which frothed vigorously, was then stirred and heated under reflux for 3 days. The suspension was then hot-filtered and the insoluble residue dried in vacuo at 70° C for 3 days to give a yellow powder (170 g). This solid was suspended in a mixture of concentrated hydrochloric acid (500 mL) and water (1 L), stirred, heated under reflux for 4 h. Hot filtration gave crude 3-nitronaphthalene-1-carboxylic acid (92.3 g), which was recrystallized from glacial acetic acid with hot filtration to remove some insoluble material to afford the title compound (36.4 g) as a cream, crystalline solid: mp 255−257 °C.

(b) 3-Amino-1-naphthalenecarboxylic Acid, Sodium Salt. A suspension of the above nitro compound (10.0 g, 0.046 mol) in ethanol (100 mL) was treated with 10% aqueous sodium hydroxide (16.6 mL, 0.046 mol) and water (20.0 mL) and stirred until all solids had dissolved. This solution was then hydrogenated over 10% (w/w) palladium-on-charcoal (2.0 g) at room temperature and 50 psi for 4 h. The solution was filtered and evaporated to afford the title compound (8.65 g, 90%) as a yellow solid: ^{1}H NMR (d_6-DMSO) δ 8.69 (d, J = 8.4 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.28 (d, J = 2.2 Hz, 1H), 7.19 (m, 1H), 7.01 (m, 1H), 6.72 (d, J = 2.2 Hz, 1H), 5.2 (br s, 2H).

(c) 3-Hydroxy-1-naphthalenecarboxylic Acid (28). A solution of the above amine (8.73 g, 0.042 mol) in water (300 mL) was treated with 2 M aqueous sulfuric acid (60.0 mL) to precipitate the free acid as a fine powder. This suspension was then stirred, cooled to 10 °C, and then slowly treated dropwise with a solution of sodium nitrite (3.03 g, 0.044 mol) in water (30.0 mL). After 30 min at 10 °C the solution was added *very slowly* dropwise to a refluxing solution of 40% aqueous sulfuric acid (1 L). Complete addition took ∼1 h. After complete addition the mixture was heated under reflux for a further 15 min and then quickly hot-filtered through a plug of glass wool to remove insoluble, charred material. The filtrate was allowed to cool, depositing the title compound (5.85 g, 74%) as yellow crystals: mp 210−212 °C; ^{1}H NMR (d_6-DMSO) δ 8.83 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7,69 (d, J = 2.0 Hz, 1H), 7.40−7.15 (m, 3H).

(d) 4-Amino-3-hydroxy-1-naphthalenecarboxylic Acid (29). A stirred solution of *p*-benzenediazonium sulfonate, prepared by the addition of sodium *p*-aminobenzenesulfonate (0.525 g, 2.7 mmol) to sodium nitrite at 0 °C, and the naphthol **28** (0.413 g, 2.2 mmol) in water (20 mL) was treated portionwise with sodium bicarbonate (2.3 g, 27.0 mmol). The resulting solution was stirred and heated at 60 °C overnight. After the solution was cooled to 50 °C, sodium hydrogen sulfite (1.05 g, 6.0 mmol) was added, and the resulting solution was stirred for 30 min at 50 °C. After being cooled to room temperature, the suspension was filtered, the filtrate evaporated to dryness, and the residue purified by flash chromatography (silica gel, 45% dichloromethane/45% ethyl acetate/10% methanol) to give the title compound (0.066 g, 15%): ^{1}H NMR (d_6-DMSO) δ 12.1 (br s, 1H), 9.45 (s, 1H), 9.06 (m, 1H), 8.08 (m, 1H), 7.37 (m, 2H), 5.89 (br s, 2H).

(e) 3-Hydroxy-4-[*N*′-(3-methyl-5-oxo-1-phenyl-1,5-dihydropyrazol-4-ylidene)hydrazino]naphthalene-1-carboxylic Acid (30a). To a stirred solution of amine **29** (0.203 g, 1.0 mmol) in ethanol (1.0 mL), ice (1.0 g), and hydrochloric acid (37%, 0.3 mL) was added a solution of sodium nitrite (0.083 g, 1.2 mmol) in water (0.2 mL). After the resulting solution was stirred at room temperature for 30 min, 5-methyl-2-phenyl-2,4-dihydropyrazol-3-one (0.174 g, 1.0 mmol) was added. Sodium bicarbonate (0.84 g, 10 mmol) was added slowly into the reaction mixture, and the resulting solution was heated at 60 °C with stirring overnight. A red precipitate was obtained by adding 3 M aqueous hydrochloric acid solution and purified by chromatography [ODS, gradient 10−90% acetonitrile/water (0.1% TFA)] to give the title compound (0.021 g, 5%): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 13.3 (br s, 1H), 11.3 (br s, 1H), 9.06 (d, J = 8.6 Hz, 1H), 8.90 (d, J = 8.8 Hz, 1H), 7.98 (s, 1H), 7.98−7.95 (m, 2H), 7.68−7.46 (m, 4H), 7.25 (m, 1H), 2.38 (s, 3H).

3-Hydroxy-4-{*N*′-[3-methyl-1-(4-methylphenyl)-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}naphthalene-1-carboxylic Acid (30b). Following the procedure of **31a**, except substituting 3-methyl-1-(4-methylphenyl)-3-pyrazolin-5-one for 5-methyl-2-phenyl-2,4-dihydropyrazol-3-one, the title compound was prepared as a red solid (0.004 g): ^{1}H NMR (d_6-DMSO) δ 14.0 (br s, 1H), 11.3 (br s, 1H), 9.06 (d, J = 8.6 Hz, 1H), 8.91 (d, J = 8.6 Hz, 1H), 8.33 (s, 1H), 7.98 (s, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.65 (m, 1H), 7.54 (m, 1H), 7.29 (d, J = 8.5 Hz, 1H), 2.38 (s, 3H), 2.34 (s, 3H).

4-{*N*′-[1-(3,4-Dimethylphenyl)-3-methyl-5-oxo-1,5-dihydropyrazol-4-ylidene]hydrazino}-3-hydroxynaphthalene-1-carboxylic Acid (30c). A methanol solution of pyrazole **15m** (0.85 g, 4.19 mmol) and tosyl azide (0.84 g, 4.19 mmol) was treated with triethylamine (0.42 g, 4.19 mmol). The reaction was stirred at room temperature for 2 h. It was concentrated, applied to a silica gel column, and eluted with ethyl acetate and hexanes to give crude 4-diazo-1-(3,4-dimethylphenyl)-3-methyl-3-pyrazolin-5-one as a yellow powder (0.5 g, 53%). A portion of this diazo compound (30.0 mg, 0.13 mmol) and the naphthol **28** (28.0 mg, 0.15 mmol) in ethanol (3.0 mL) were treated with triethylamine (8 drops) and stirred at room temperature for 96 h. The mixture was evaporated and treated with ethyl acetate and 3 M aqueous hydrochloric acid. The organic layer was dried and evaporated to afford the title compound **30c** (20.0 mg, 37%) as a red solid: ^{1}H NMR

(d_6-DMSO) δ 14.1 (br s, 1H), 11.3 (br s, 1H), 9.6 (br s, 1H), 9.04 (d, J = 8.4 Hz, 1H), 8.86 (d, J = 8.4 Hz, 1H), 7.99 (s, 1H), 7.76 (s, 1H), 7.68 (m, 2H), 7.42 (m, 1H), 7.19 (d, J = 8.1 Hz, 1H), 2.37 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H).

Biology. Luciferase Assay.[32−35] The BAF3-3B5 reporter cell line was isolated after cotransfection of the murine, parental BAF3 cell line with two plasmids, one an expression plasmid directing constitutive expression of the human TPO receptor as well as neomycin gene and the second a luciferase reporter construct consisting of three tandem copies of the IRF-1 element upstream of a minimal tk promoter. Transfected cells were then selected for G418 resistance, and the "3B5" clone was eventually chosen for screening. BAF3-3B5/TPO-Rluc cells (1×10^5/mL) (starved of IL-3 overnight) in growth medium containing fetal bovine serum (FBS) (0.5% v/v) and $ZnCl_2$ (30 μM) were incubated with compounds (0.32% DMSO final concentration) or rhTPO at 37 °C (5% CO_2, 95% relative humidity) for 3 h. Luciferase activity was recorded by standard methods[32−35] using a Dynatech model 1000 luminometer. The mean luciferase value over control expressed as a percentage of that induced by maximal TPO concentration for duplicate assays was plotted vs concentration, and EC_{50} values were calculated.

Proliferation Assay.[56] UT7/TPO cells [grown in IMDM containing FBS (10%, v/v), glutamine, and rhTPO (R&D Systems, 100 mg/mL)] were starved overnight in IMDM, supplemented with FBS (10% v/v) and glutamine (no TPO), prior to assay. UT7/TPO cells (0.2×10^6 cells/mL) were resuspended in the starving medium above and then incubated with compounds (0.32% DMSO final concentration) or rhTPO at 37 °C (5% CO_2, 95% relative humidity) for 72 h. After centrifugation, the cell pellet total DNA content was measured using a BrdU proliferation kit (Boehringer Mannheim, catalog no. 1647229). The mean proliferation value over control expressed as a percentage of that induced by maximal TPO concentration for duplicate assays was plotted vs concentration, and EC_{50} values were calculated.

CD34$^+$ Differentiation Assay. Human bone marrow CD34$^+$ cells prepared from light density marrow cells (2×10^6 cells/mL) in IMDM containing fetal calf serum (FCS) (20% v/v) and recombinant human stem cell factor (rhSCF) (100 ng/mL) were incubated with TPO (maximal TPO 100 ng/mL) or a sample for 10 days at 5% CO_2 and 4% O_2. Each sample was stained with FITC-anti-IgG1 isotype control and FITC-anti-CD41 (Pharmingen clone SZ22) and fixed in formaldehyde. Cells were analyzed on a Becton-Dickinson FACScan flow cytometer. The percent of CD41 cells for each sample was calculated by subtracting the isotype control positive from the CD41 positive cells. Data are expressed as a percent of the TPO max = (% CD41 sample − % CD41 rhSCF)/(% CD41 TPO − % CD41 rhSCF).

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