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Synthesis and biological evaluation of oxoindolin-3-ylidene ethyl benzothiohydrazides as non-peptide TPO mimics

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ABSTRACT

A novel series of oxoindolin-3-ylidene ethyl benzohydrazides were designed, synthesized, and identified as small molecule agonists of thrombopoietin (TPO) receptor c-mpl. Sulfur–oxygen exchange in oxoindolin-3-ylidene ethyl benzohydrazides was found to improve their agonistic activities. Several oxoindolin-3-ylidene ethyl benzothiohydrazides have been identified as full agonists of c-mpl.

Platelets are essential in the process of blood clotting and repair of damaged blood vessels. Thrombocytopenia, or low circulating platelet levels, can be a debilitating condition associated with a range of medical disorders, including platelets immunogenicity (ITP),[1] liver dysfunctions, drug treatments (IFN,[2] heparin[3]), and viral infections (HIV,[4] hepatitis C[5]), and is also a common side effect in cancer patients receiving intensive chemotherapy treatments. Current methods to manage thrombocytopenia includes corticosteroids, intravenous immunoglobulin, anti-D immunoglobulin, rituximab, vinca alkaloids, azathioprine, cyclophosph-amide, mycophenolate mofetil, cyclosporine, as well as splenectomy,[6] all of which suffer from serious side effects or low efficacy.

Thrombopoietin (TPO), a cytokine produced primarily by the liver and kidney, regulates platelet production by stimulating proliferation and differentiation of hematopoietic stem cells, megakaryocytic progenitor cells, and megakaryocytes via activation of its receptor, c-mpl.[7–11] Continuing understanding of the structure and function of TPO led to the development of the first generation thrombopoietic growth factors, including rhTPO and PEG-rHuMGDF. Clinical study of both of them, unfortunately, were halted because of the risk of antigenicity.[12]

Despite the problem of autoantibody formation, the results of the clinical trials with the first generation TPO molecules provided a stimulus for continuing the search and development of non-immunogenic TPO molecules, namely, the second generation thrombopoietic agonists. Several agents have progressed to clinical trials, and Eltrombopag and AMG-531 have reached the US market in 2008.

AMG-531, a TPO mimetic peptide, is a 60 kDa molecule that consists of disulfide-bonded human IgG1 heavy chain constant regions with two identical peptide sequences linked covalently through a polyglycine bridge. AMG-531 was administered intravenously or subcutaneously and showed remarkable ability to promote platelet counts in clinical trials.[13] Eltrombopag, however, is a non-peptide hydrazone small molecule, with a molecular weight of 564 kDa.[13] Eltrombopag is the first and so far the only one oral treatment for thrombocytopenia.

According to Duffy et al., a heteroatom metal chelate in the central portion of the molecule is indispensable for its TPO receptor agonist activity.[14] Nissan researchers have reported a novel thiophene carbohydrazone compound NIP-004 (Fig. 1) as human TPO receptor (c-mpl) activator.[15]

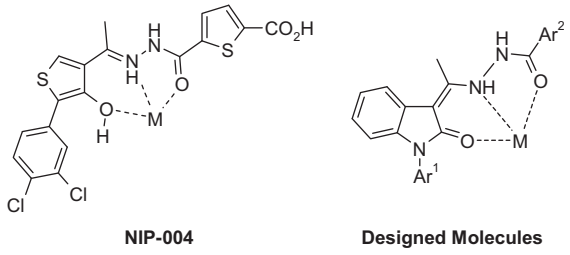

Figure 1. NIP-004 and designed oxoindolin-3-ylidene ethyl benzohydrazides as metal chelators.

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Table 1
Chemical structures and bioactivities of **5aa–la**

Compound	Ar^1	R	Ar^2	BaF3/TPOR [a] (%)	
				150 nM	300 nM
5aa	4-Me-Ph	H	4-CO_2H-Ph	11.5	57.6
5ab	4-Me-Ph	H	3-CO_2H-Ph	6.1	45.2
5ac	4-Me-Ph	H	2-CO_2H-Thiophene-5-yl	10.5	39.6
5ba	4-Me-Ph	5-F	4-CO_2H-Ph	38.8	147.1
5bb	4-Me-Ph	5-F	3-CO_2H-Ph	23.3	60.2
5ca	3-Me-Ph	H	4-CO_2H-Ph	20.9	90.2
5cb	3-Me-Ph	H	3-CO_2H-Ph	10.3	47.5
5cc	3-Me-Ph	H	2-CO_2H-Thiophene-5-yl	8.2	56.8
5da	4-Et-Ph	H	4-CO_2H-Ph	13.6	70.2
5db	4-Et-Ph	H	3-CO_2H-Ph	5.8	45.3
5ea	3,4-DiMe-Ph	H	4-CO_2H-Ph	16.2	82.3
5eb	3,4-DiMe-Ph	H	3-CO_2H-Ph	7.2	61.5
5ec	3,4-DiMe-Ph	H	2-CO_2H-Thiophene-5-yl	21.3	55.9
5fa	Ph	H	4-CO_2H-Ph	5.5	39.5
5fb	Ph	H	3-CO_2H-Ph	4.7	12.1
5ga	5-Indanyl	H	4-CO_2H-Ph	19.3	68.4
5gb	5-Indanyl	H	3-CO_2H-Ph	8.6	33.9
5ha	5-Indanyl	5-F	4-CO_2H-Ph	21.5	96.3
5hb	5-Indanyl	5-F	3-CO_2H-Ph	10.2	29.5
5ia	4-Me-Ph	5-NHAc	4-CO_2H-Ph	12.5	31.0
5ib	4-Me-Ph	5-NHAc	3-CO_2H-Ph	16.8	28.2
5ja	4-Me-Ph	5-Pyrrol-1-yl	4-CO_2H-Ph	8.1	19.6
5jb	4-Me-Ph	5-Pyrrol-1-yl	3-CO_2H-Ph	8.2	13.8
5ka	4-Me-Ph	4-F	4-CO_2H-Ph	26.9	81.3
5la	4-Me-Ph	6-F	4-CO_2H-Ph	35.5	100.2

[a] The growth rate of BaF3/TPOR in the presence of each compound at 150 or 300 nM, expressed by taking the value observed in the presence of 10 ng/ml TPO as 100% standard.

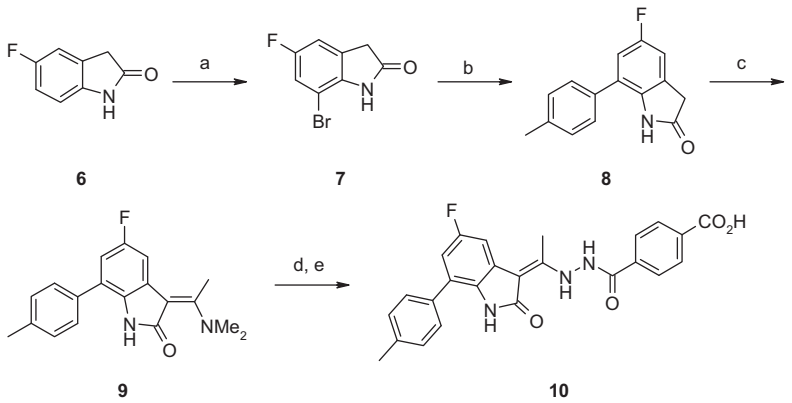
Oxoindolin-3-ylidenes were also reported as good metal chelators.[16] Replacement of the 2-(3,4-dichlorophenyl)-thiophene-3-ol group in NIP-004 with 1-aryl-indolin-2-ones results in a new group of fine metal chelators (Fig. 1), with the potential to be TPO mimicking compounds of considerable activity. Herein, we report our efforts on synthesis and evaluation of oxoindolin-3-ylidene ethyl benzothiohydrazides as TPO receptor agonists. Several compounds were found to be full agonists of TPOR in our in vitro model.

Ethoxycarbonyl substituted benzoic acids or thiophene-2-carboxylic acids were reacted with *tert*-butyl *N*-aminocarbamate, followed by de-protection of the Boc group with TFA to give the corresponding benzohydrazides, which were reacted with 3-dimethylamino-ethylidene-oxindoles **4a–l** to afford the coupling products. A simple sodium hydroxide treatment afforded the corresponding carboxylic acid products **5aa–la**.[17] 3-Dimethylamino-ethylidene-oxindoles **4a–l** were obtained from commercially available oxindoles through reported procedures.[18]

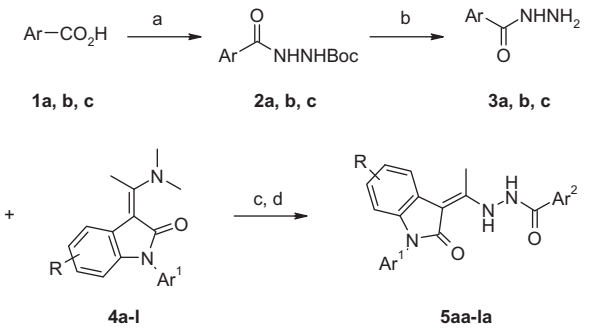
Potency of compounds **5aa–la** as TPO receptor agonists were tested in a TPO dependent BaF3/TPOR cell line, which was established by introducing human TPO receptor into BaF3 cells.[19] The structures of **5aa–la** were outlined in Table 1.

Most of the above compounds were found to be only weakly active or inactive at all in the BaF3/TPOR cell line, except for compounds **5ba**, **5ha**, and **5la**, which appeared to be full agonist of TPOR at 300 nM in our assay. Compounds **5ba**, **5ha**, and **5la** are all benzoic acids, while all thiophene-2-carboxylic acid compounds (**5ac**, **5cc**, and **5ec**) exhibited weak TPO activity. Lack of TPO mimicking potency of compound **5bb** indicates the positive effect of the 4-carboxylic acid substitution on the right phenyl ring. The 5-F substitution seems to be optimal, considering the less potent compounds **5aa**, **5ka**, and **5la**. Bulky substituents on the oxoindoline group were not tolerated (**5ia–jb**).

Shifting of the 4-methylphenyl group in **5ba** to 7-position of the oxindole group was proved to be futile. Compound **10** (Scheme 2) was synthesized and found to be inactive in the BaF3/TPOR assay,



Scheme 2. Synthesis of 7-substituted oxindole compound **10**. Reagents and conditions: (a) NBS, CH_3CN, rt, overnight, 44%; (b) *p*-tolylboronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O (1:1), reflux, 8 h, 62%; (c) $MeC(OMe)_2NMe_2$, $CHCl_3$, reflux, 8 h, 95%; (d) **3a**, EtOH, AcOH (cat.), reflux, 6 h, 90%; (e) NaOH, MeOH, H_2O, reflux, 3.5 h, 95%.



Scheme 1. Synthesis of substituted benzohydrazides **5aa**–**la**. Reagents and conditions: (a) DCC, NEt_3, DMAP, rt, 2 h, 67–90%; (b) TFA, CH_2Cl_2, rt, 2 h, 75–92%; (c) EtOH, AcOH (cat.), reflux, overnight, 55–85%; (d) NaOH, MeOH, rt, >90%.

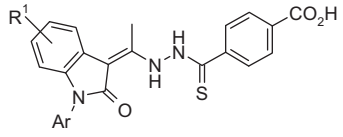


Figure 2. Chemical structures of compounds **11a**–**e**.

Table 2
TPO receptor agonist activity of benzothiohydrazides **11a**–**e**

Compound	Ar	R^1	EC_{50} (μM) [a]
11a	3,4-DiMe-Ph	H	0.082
11b	5-Indanyl	H	>0.2
11c	4-Me-Ph	5-F	0.044
11d	3,4-DiMe-Ph	5-F	0.103
11e	5-Indanyl	5-F	>0.2

[a] Average results of three experiments. EC_{50} of Eltrombopag was found to be 120 nM under the same condition.

indicating suitable aryl substitution on the oxoindoline ring to be vital for TPO receptor activating potency.

Sulfur is generally accepted as a stronger chelator than oxygen.[20] Considering the importance of chelation in thrombopoietic activities, sulfur–oxygen exchange in compound **5ba** may result in stronger chelation and thus higher TPOR agonist potency. Boc protected benzohydrazides **2a**,**b** were treated with Lawensson's reagents to give the corresponding benzothiohydrazides, which underwent similar reaction sequence as **2a**,**b** in Scheme 1, providing oxoindolin-3-ylidene benzothiohydrazides **11a**–**e** (Fig. 2) in good overall yields.[17] Structures and thrombopoietic activities of **11a**–**e** were outlined in Table 2.

Compounds **11a**, **11c**, and **11d** were found to be full agonists of human TPO receptor c-mpl, among which **11c** was the most potent compound. Compounds **11b** and **11e** surprisingly exhibited only weak thrombopoietic activities, without any obvious explanation. Among the active compounds, compound **11c** with 4-methyl phenyl and a 5-fluoro substitution on the oxindole structure was again the most potent compound, in line with what was seen in the oxo-series compounds.

In summary, sulfur–oxygen exchange in oxoindolin-3-ylidene ethyl benzohydrazides resulted in dramatic improvement in their thrombopoietic activities. Members of these compounds were found to be full agonists of human c-mpl in our in vitro assay. In vitro and in vivo pharmacokinetic and efficacy studies of selected compounds are under way. This may provide an alternative route to develop novel therapeutic agents to treat thrombocytopenia.

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