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# Novel indoline-1- or 3,4-dihydroquinoline-1(2*H*)-substituted carbothiohydrazides as TPO receptor agonists

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ABSTRACT

Synthesis and evaluation of novel series of indoline-1- or 3,4-dihydroquinoline-1(2*H*)-substituted carbothiohydrazide or carbohydrazide based small molecule compounds as thrombopoietin (TPO) receptor agonists are reported. Members of these compounds have been identified as full agonists of human c-mpl in BaF3/TPOR cell line. Indoline-1-carbohydrazide **9b** exhibited reasonable pharmacokinetic profile.



Thrombocytopenia, or low circulating platelet levels, is a frequent finding in a range of medical disorders, such as aplastic anemia,[1] viral infections (HIV,[2] hepatics C[3]), myelodysplasia,[4] idiopathic thrombocytopenia purpura(ITP),[5] and liver dysfunctions.

Thrombopoietin (TPO) is the key cytokine involved in thrombopoiesis, and is the endogenous ligand for the thrombopoietin receptor (TPOR) that is expressed on the surface of megakaryocytes, and megakaryocytic precursors.[6] Binding of TPO to its receptor triggers the activation of the JAK-STAT pathway, leading to changes in gene expression that promote progression along the megakaryocytic pathway, ultimately leading to the release of platelets into the peripheral circulation.

Structural elucidation of TPO and its receptor c-mpl resulted in prompt development of rhTPO and pegylated-rHuMGDF, clinical trial of which, unfortunately, were stopped for immunogenic drawbacks.[7]

Recent research has resulted in development of oral non-peptide TPO receptor agonists to treat thrombocytopenia. Several non-peptide TPO mimetics (Eltrombopag,[8] AKR-501,[9] LGD-4665[10]) have entered human clinical trials, and one of them, eltrombopag, has reached the US market. Non-peptide TPO mimetics are thought to bear obvious benefits, such as a lower cost of production, an easier route of administration (orally available), outpatient treatment, and non-immunogenicity, etc.[11] There are still great opportunity for alternative nonpeptidyl orally administered agents.

Some thio-urea compounds with good in vitro thrombopoietic activities has been disclosed by Nissan researchers, while no further evaluation was reported thereafter.[12] We have reported a series of oxoindolin-3-ylidene ethyl benzothiohydrazides as potent TPO receptor agonists.[13] Herein we report another series of novel indoline-1- or 3,4-dihydroquinoline-1(2*H*)-substituted carbothiohydrazide or carbohydrazide based compounds as thrombopoietin receptor agonists.

Commercially available methyl indoline-5-carboxylate (**1a**) or methyl 1,2,3,4-tetrahydro-6-quinolinecarboxylate (**1b**) were treated with 1,1′-thiocarbonyldiimidazole in THF, followed by hydrazine hydrate, giving thiosemicarbazides **3a**, **b** in good yields. Reaction of **3a**, **b** with 3-dimethylamino-ethylidene-oxindoles **4a–e**, followed by a simple sodium hydroxide treatment afforded the final products **5a–g** in moderate to good yields.[14] (Scheme 1) 3-dimethylamino-ethylidene-oxindoles **4a–e** were obtained from commercially available oxindoles through reported procedures.[15a,b]

Potency of compounds **5a–g** as TPO receptor agonists were tested in a TPO dependent BaF3 cell line by introducing human TPO receptor into BaF3 cells.[16] Structures and bioactivities of **5a–g** were outlined in Table 1.

Compounds **5a**, **5b**, **5c**, and **5g** were found to be full agonists of TPO receptor in the BaF3/TPOR cell line.

Encouraged by the results of **5a–g**, their pyrazolone analogues were also synthesized and tested. 4-[1-(Dimethylamino)ethylidene]-2-aryl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-ones **6a–b** were reacted with thiosemicarbazides **3a–b**, followed by a simple sodium hydroxide treatment afforded the final products **7a–d** in moderate to good yields (Scheme 2). 4-[1-(Dimethylamino)ethylidene]-2-aryl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-ones **6a–b** were obtained from commercially available pyrazolones through reported procedures.[17]

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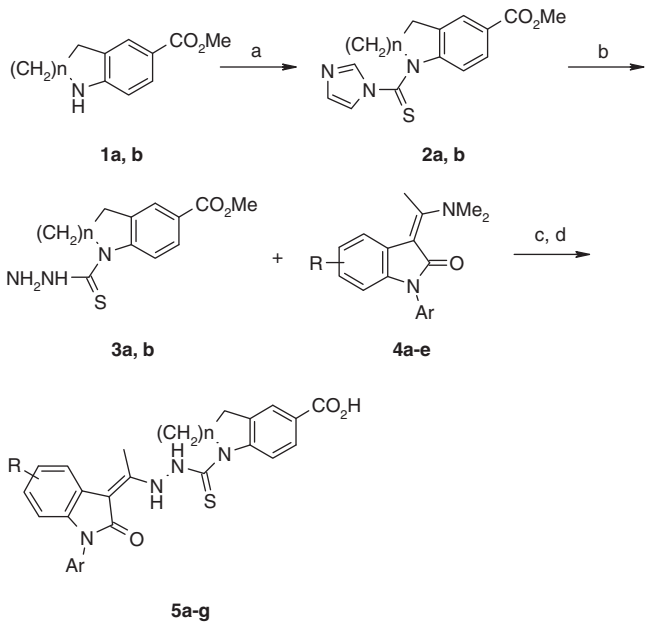
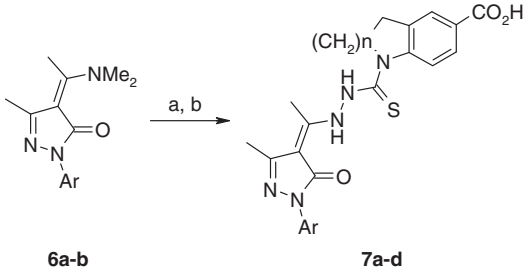


**Scheme 1.** Synthesis of carbothiohydrazides **5a**–**g**. Reagents and conditions: (a) 1,1′-thiocarbonyldiimidazole, THF, rt, 2 h, 41–55%; (b) $NH_2NH_2{\cdot}H_2O$, THF, rt, 1 h, 70–75%; (c) EtOH, AcOH(cat.), 60 °C, 2 h, 60–80%; (d) NaOH, MeOH, rt, >90%.

**Table 1**
Structures and activities of **5a**–**g**

| No. | *n* | Ar | R | BaF3/TPOR Screening[a] | |
|---|---|---|---|---|---|
| | | | | 150 nM | 300 nM |
| **5a** | 1 | 3,4-Dimethylphenyl | H | 72.4% | 137.8% |
| **5b** | 1 | 4-Methylphenyl | H | 47.8% | 107.3% |
| **5c** | 1 | 4-Methylphenyl | 5-F | 78.1% | 122.4% |
| **5d** | 1 | 5-Indanyl | H | 14.3% | 75.8% |
| **5e** | 1 | 5-Indanyl | 5-F | 3.2% | 10.6% |
| **5f** | 2 | 3,4-Dimethylphenyl | H | 30.9% | 33.4% |
| **5g** | 2 | 4-Methylphenyl | H | 47.3% | 101.4% |

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



**Scheme 2.** Synthesis of carbothiohydrazides **7a**–**d**. Reagents and conditions: (a) **3a**,**b**, EtOH, AcOH(cat.), 60 °C, 1 h, 65–80%; (b) NaOH, MeOH, rt, >90%.

As shown in Table 2, compounds **7a**–**d** were found to be highly active in the BaF3/TPOR cell line. $EC_{50}$ of the most potent compound **7b** was 3 nM. Ring size of benzene fused heterocycle exhibited clear effect on thrombopoietic activities of the compounds. Indoline-1-carbothiohydrazides **7a**, **b** are 10 times more potent than 3,4-dihydroquinoline-1(2*H*)-carbothiohydrazides **7c**, **d**.

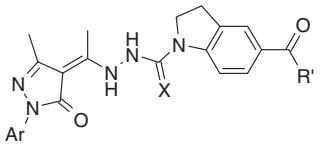
Substitutions on indolino-benzene ring of **7a** were varied by displacement of the carboxylic acid by amides, which were well tolerated, retaining good TPO receptor agonist activities. Thus,

**Table 2**
Structures and activities of compounds **7a**–**d**

| No. | *n* | Ar | $EC_{50}$[a] (nM) |
|---|---|---|---|
| **7a** | 1 | 3,4-Dimethylphenyl | 6 |
| **7b** | 1 | 5-Indanyl | 3 |
| **7c** | 2 | 3,4-Dimethylphenyl | 64 |
| **7d** | 2 | 5-Indanyl | 35 |

[a] Values are means of three experiments. $EC_{50}$ of Eltrombopag was found to be 120 nM under the same condition.

**Table 3**
Structures and activities of compounds **8a**–**c** and **9a**–**b**



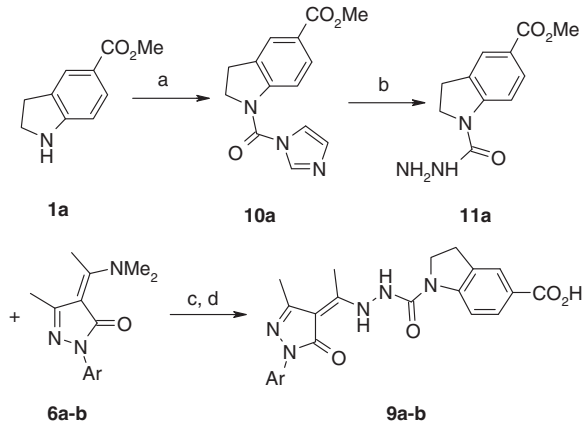
| No. | X | Ar | R′ | $EC_{50}$[a] (nM) |
|---|---|---|---|---|
| **8a** | S | 3,4-Dimethylphenyl | –NHMe | 22 |
| **8b** | S | 3,4-Dimethylphenyl | Morpholino- | 31 |
| **8c** | S | 3,4-Dimethylphenyl | –NHEt | 26 |
| **9a** | O | 3,4-Dimethylphenyl | –OH | 42 |
| **9b** | O | 5-Indanyl | –OH | 46 |

[a] Values are means of three experiments.

reaction of **7a** with various amines gave the corresponding amides **8a**–**c**, and their structures and bioactivities were outlined in Table 3. Sulfur–oxygen exchange in **7a** and **7b** resulted in carbohydrazide compounds **9a** and **9b** respectively, also with good thrombopoietic activities retained. Synthesis of Compounds **9a** and **9b** were summarized in Scheme 3.

Selected compounds were converted into their ethanolamine salts and administered orally at 5 mg/kg dosage respectively to rats. While most thio-urea compounds, including **7a**/**7b**, were found only moderately absorbed (data not shown), compounds **9a** and **9b** did exhibited reasonable pharmacokinetic profiles, as shown in Table 4.

Comparing with **9a**, compound **9b** was well absorbed orally in rats. AUC of **9b** was four times higher than that of **9a**, indicating a favorable effect of the indane substitution. Clearance half life of both **9a** and **9b** were less than 2 h, which may account for the low plasma exposures of both compounds. Efforts must be taken



**Scheme 3.** Synthesis of carbohydrazides **9a**–**b**. Reagents and conditions: (a) 1,1′-carbonyldiimidazole, THF, rt, 2 h, 62%; (b) $NH_2NH_2{\cdot}H_2O$, THF, rt, 1 h, 81%; (c) EtOH, AcOH(cat.), 60 °C, 2 h, 63–70%; (d) NaOH, MeOH, rt, >90%.

**Table 4**
Pharmacokinetic properties of **9a**, **9b** ethanolamine salts

| Compd | Clz/F (L/h/kg) | $T_{1/2}$ (h) | $AUC_{0-t}$ (ng h/mL) | $C_{max}$ (ng/mL) |
|---|---|---|---|---|
| **9a** | 6.78 ± 3.49 | 1.86 ± 0.27 | 923 ± 504 | 859 ± 336 |
| **9b** | 1.23 ± 0.42 | 1.48 ± 0.30 | 4460 ± 1640 | 3553 ± 884 |

Clz/F, apparent total plasma clearance; $T_{1/2}$, half life; AUC, area under the plasma concentration–time; $C_{max}$, peak plasma concentration.

to improve the metabolic stability of such molecules and reduce body clearance to obtain better pharmacokinetic profile.

In summary, we have developed indoline-1- and 3,4-dihydroquinoline-1(2*H*)-substituted carbothiohydrazide as well as carbohydrazide based small molecule compounds as TPO mimetics. Several compounds were found to be full agonists of TPO receptor in the BaF3/TPOR cell line, although their pharmacokinetic properties will require further optimization. Efforts to improve PK/PD profile of such compounds are under way. This may provide an alternative route to develop novel therapeutic agents to treat thrombocytopenia.

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## References and notes

1. Jenkins, J. M.; Williams, D.; Deng, Y.; Uhl, J.; Kitchen, V.; Collins, D.; Erickson-Miller, C. L. *Blood* **2007**, *109*, 4739.
2. Mannucci, P. M.; Gringeri, A. *Ann. Ital. Med. Int.* **2000**, *15*, 20.
3. Espanol, I.; Gallego, A.; Enriquez, J.; Rabella, N.; Lerma, E.; Hernandez, A.; Pujol-Moix, N. *Hepatogastroenterology* **2000**, *47*, 1404.
4. Moody, J. L.; Xu, L.; Helgason, C. D.; Jirik, F. R. *Blood* **2004**, *103*, 4503.
5. Andersson, J. *Acta Paediatr. Suppl.* **1998**, *424*, 61.
6. Kuter, D. J. *Annu. Rev. Med.* **2009**, *60*, 193.
7. Kuter, D. J.; Begley, C. G. *Blood* **2002**, *100*, 3457.
8. Skirvin, J. A. *Formulary* **2009**, *43*, 356.
9. Fukushima-Shintani, M.; Suzuki, K.; Iwatsuki, Y.; Abe, M.; Sugasawa, K.; Hirayama, F.; Kawasaki, T.; Nakahata, T. *Eur. J. Haematol.* **2009**, *82*, 247.
10. Tomilero, A.; Moral, M. A. *Methods Find. Exp. Clin. Pharmacol.* **2008**, *30*, 543.
11. Duffy, K. J.; Darcy, M. G.; Delorme, E.; Dillion, S. B.; Eppley, D. F.; Erickson-Miller, C.; Giampa, L.; Hopson, C. B.; Huang, Y.; Keenan, R. M.; Lamb, P.; Leong, L.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Shah, R.; Shaw, T. N.; Smith, H.; Kenneth, C. S.; Tian, S. –S.; Tyree, C.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2001**, *44*, 3730.
12. Miyaji, K.; Iwamoto, S.; Shigeta, Y.; Hirokawa, Y.; Owada, S.; Nakamura, T.; Ishiwata, N. PCT Int. Appl. WO 2007142308 A1, 2007.
13. Tang, P. C.; Lu, H. J.; Chen, Y. Q.; Wang S. L.; Zheng, H.; Wang, L. *Bioorg. Med. Chem. Lett.* **2010**, in submit.
14. All new compounds were characterized by $^1$H NMR, $^{13}$C NMR, MS and IR. *Data for compound* **5a**: $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) $\delta$ 11.68 (br, 1H), 11.48 (br, 1H), 8.76 (d, 1H, $J$ = 8.8 Hz), 7.88 (s, 1H), 7.82 (d, 1H, $J$ = 8.8 Hz), 7.54 (d, 1H, $J$ = 5.6 Hz), 7.33 (d, 1H, $J$ = 8.0 Hz), 7.22 (d, 1H, $J$ = 2.0 Hz), 7.15 (dd, 1H, $J_1$ = 8.0 Hz, $J_2$ = 2.0 Hz), 7.00–7.05 (m, 2H), 6.77–6.82 (m, 1H), 4.39 (t, 2H, $J$ = 8.4 Hz), 3.23 (t, 2H, $J$ = 8.4 Hz), 2.50 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$, ppm) $\delta$ 167.60, 167.50, 167.36, 147.37, 137.99, 137.87, 136.08, 134.61, 133.03, 132.41, 130.67, 129.11, 128.38, 128.00, 126.49, 126.09, 124.75, 124.42, 123.27, 121.83, 119.23, 116.40, 108.75, 53.07, 26.91, 19.86, 19.55, 15.60; MS (ESI) $m/z$: 499.6 $[M+1]^+$; IR (KBr): 3434.5, 2922.4, 1680.7, 1607.3, 1505.6, 1449.3, 1370.9, 1279.9, 1255.1, 1198.3, 1107.1, 951.6, 914.6, 874.4, 819.6, 773.0, 695.6, 610.9, 444.1 cm$^{-1}$. *Data for compound* **7a**: $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) $\delta$ 8.62 (d, 1H, $J$ = 6.8 Hz), 7.79–7.81 (m, 2H), 7.70 (s, 1H), 7.64 (d, 1H, $J$ = 8.0 Hz), 7.19 (d, 1H, $J$ = 8.0 Hz), 4.38 (t, 2H, $J$ = 8.4 Hz), 3.18 (t, 2H, $J$ = 8.4 Hz), 2.51 (s, 3H), 2.44 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H); MS (ESI) $m/z$: 464.6 $[M+1]^+$. *Data for compound* **9a**: $^1$H NMR (400 MHz, DMSO-$d_6$, ppm) $\delta$ 12.31 (br, 1H), 9.70 (br, 1H), 7.90–7.92 (m, 1H), 7.80–7.84 (m, 3H), 7.70–7.74 (m, 1H), 7.23 (d, 1H, $J$ = 8.0 Hz), 4.13 (t, 2H, $J$ = 8.4 Hz), 3.25 (t, 2H, $J$ = 8.4 Hz), 2.52 (s, 3H), 2.50 (s, 3H), 2.44 (s, 3H), 2.36 (s, 3H); MS (ESI) $m/z$: 448.5 $[M+1]^+$.
15. (a) Sarges, R.; Howard, H. R.; Koe, B. K.; Weissman, A. *J. Med. Chem.* **1989**, *32*, 437; (b) Philips, D. P.; Hudson, A. R.; Nguyen, B.; Lau, T. L.; McNeill, M. H.; Dalgard, J. E.; Chen, J. H.; Penuliar, R. J.; Miller, T. A.; Lin, Z. *Tetrahedron Lett.* **2006**, *47*, 7137.
16. Wild-type BaF3 cell line was transfected with an EX-B0010-M02 plasmid containing TPO receptor gene and neomycin, screened by G418 (Gibco, US) to get the stable monoclonal BaF3-TPOR cell line. In a 96-well plate, to each well was added 100 μl of the cell suspension, the blank control, negative control, rhTPO and the test compound of different concentrations. All measurements were made in triplicate. The plate was incubated for 24 h at 37 °C in 5% $CO_2$. 10 μl/well of CCK-8 was added and the plate was cultured for another 4 h. $OD_{450}$ value was recorded with a VICTOR3 (Perkin–Elmer 1420) instrument, and $EC_{50}$ was calculated with Origin 7.0.
17. Tang, P. C.; Lu, H.; Chen, Y.; Song, P.; Wang, S. PCT Int. Appl. WO 2009103218 A1, 2009.