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Triterpenoid Hydroxamates as HIF Prolyl Hydrolase Inhibitors

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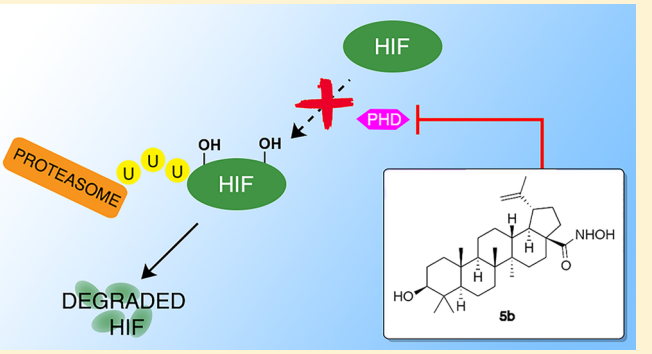
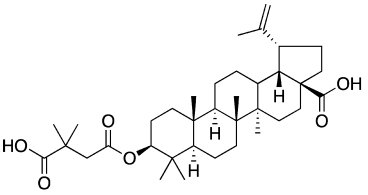
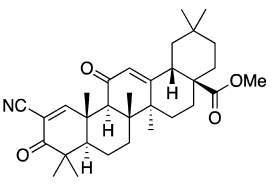
Supporting Information

ABSTRACT: Pentacyclic triterpenoid acids (PCTTAs) are pleiotropic agents that target many macromolecular endpoints with low to moderate affinity. To explore the biological space associated with PCTTAs, we have investigated the carboxylate-to-hydroxamate transformation, discovering that it de-emphasizes affinity for the transcription factors targeted by the natural compounds (NF-κB, STAT3, Nrf2, TGR5) and selectively induces inhibitory activity on HIF prolyl hydrolases (PHDs). Activity was reversible, isoform-selective, dependent on the hydroxamate location, and negligible when this group was replaced by other chelating elements or O-alkylated. The hydroxamate of betulinic acid (**5b**) was selected for further studies, and evaluation of its effect on HIF-1α expression under normal and hypoxic conditions qualified it as a promising lead structure for the discovery of new candidates in the realm of neuroprotection.



Pentacyclic triterpenoids are a class of isoprenoids derived from squalene oxide by cascade cyclization, with oleanane, ursane, and lupane being their biogenetically earliest and more widespread skeletal types.[1] Oxidation of one exocyclic methyl to a carboxylate is the most common postcyclizative transformation of pentacyclic triterpenoids, and the resulting acids (PCTTAs, pentacyclic triterpenoid acids) can be accumulated in large amounts in resins, exudates, and various living plant tissues.[1] Compared to steroids, pentacyclic triterpenoids have long been considered unattractive in terms of bioactivity, as remarked by upon the Nobel laureate Leopold Ruzicka (1887−1976), who used to compare them to "the devices in a gym, where monkeys "(unexperienced students)" learn to crawl before climbing the vertiginous heights of the steroids".[2] On the other hand, the easy availability of large amounts of the archetypal PCTTA by isolation, the chemical diversity associated with their structures, and their multitarget biological profiles have not gone unnoticed in the biomedical community, spurring drug discovery campaigns aimed at potentiating the activity of PCTTA leads or, alternatively, using their rigid scaffold to recognize specific protein surfaces. The antiviral agent bevirimat (BMS-955176, **1**)[3] and the ultrapotent antioxidant inflammation modulator bardoxolone methyl (**2**)[4] represent the clinically more advanced semisynthetic PCTTA derivatives generated in these efforts.





Natural PCTTAs can target many macromolecular endpoints with low to moderate affinity.[5] To improve potency, the most common strategy has been to implant biologically "sticky" functional groups on the lipophilic triterpenoid scaffold, as exemplified by the development of bardoxolone methyl (**2**) from oleanolic acid (**3a**), for which the potency could be increased several orders of magnitude by the insertion of a cyanoacrylate Michael acceptor element in ring A, and further modulated by changes in ring C.[4] Inspired by the success of this approach, we have attempted to replicate its

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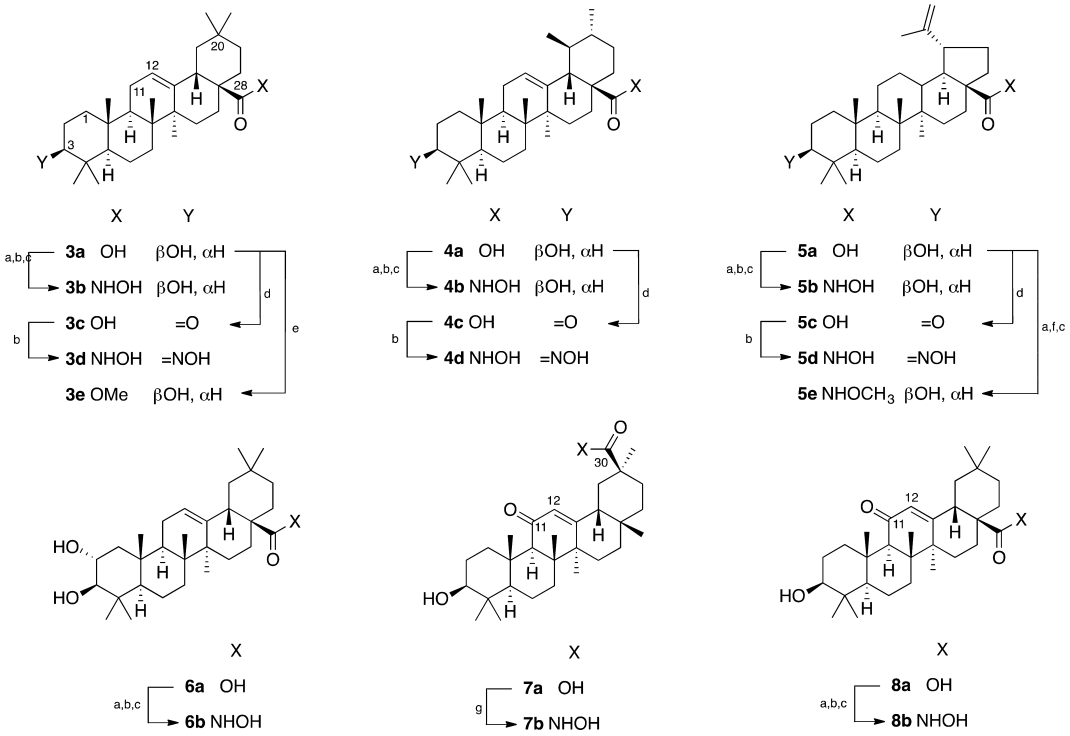
Scheme 1. Synthesis of First-Generation PCTTA Hydroxamates and Oxyminohydroxamates[a]



[a](a) Ac_2O, DMAP, pyridine (quantitative); (b) oxalyl chloride, CH_2Cl_2 40 °C then $NH_2OH{\cdot}HCl$, pyridine 40 °C (38−70%); (c) NaOH 4 M, THF/MeOH, 1:1, 40 °C (50−60%); (d) Jones reagent, acetone/EtOAc, 5:1 (nearly quantitative); (e) K_2CO_3, DMS, DMF (98%); (f) oxalyl chloride, CH_2Cl_2 40 °C then $NH_2OCH_3{\cdot}HCl$, pyridine 40 °C (38−70%); (g) $NH_2OH{\cdot}HCl$, TEA, T3P, CH_3CN (64%). Ac_2O, acetic anhydride; DMAP, 4-dimethylaminopyridine, Jones reagent sulfuric acid-chromium trioxide mixture; DMS, dimethylsulfate; DMF, dimethylformamide; TEA, triethylamine.

"dock-and-bind"[6] strategy to discover new modulators of the hypoxia-inducible factors (HIFs), as defined in their 1α (HIF-1α) and 2α (HIF-2α) isoforms.

HIF-1α and HIF-2α are transcription factors stabilized by a cellular low oxygen status (hypoxia)[7] that regulates the expression of a host of genes for which the products are involved in biological processes as diverse as erythropoiesis, angiogenesis, vascular tone, and immunity.[7] Molecular oxygen controls the cellular stability of HIF-1α and HIF-2α via HIF prolyl hydroxylases (PHDs), a class of iron-containing dioxygenases that, in the presence of molecular oxygen and 2-oxoglutarate, hydroxylate HIF-1α and HIF-2α, inducing their ubiquinitation by an E3-ubiquitin ligase and degradation by the 26S proteasome.[7] Several lines of evidence suggest that the HIF-1α/HIF-2α stabilization induced by pharmacological inhibition of PHDs may be of clinical relevance for the treatment of ischemic and inflammatory conditions[7] as well as neurological disorders.[8] Thus, HIF-1α upregulates the vascular endothelial growth factor (VEGF) and erythropoietin (EPO), both showing neuroprotective activity in different animal models of disease, including Huntington's disease.[9] Efforts to discover small-molecule PHD inhibitors have led to FG-2216 (FibroGen)[10] and roxadustat (FG-4592), with the latter being currently in phase III clinical trials for the treatment of renal anemia in patients with end-stage kidney disease.[11] GlaxoSmithKline, Bayer, Japan Tobacco Inc., and Akebia Therapeutics Inc. are some of the other industrial players in the developing of oral PHD inhibitors,[12] attesting to the interest in this area outside academia and basic research.

The HIF pathway is sensitive to chelators. Thus, the trihydroxamate siderophore desferrioxamine B (DFX) blocks PHD activity by forming a very stable hexadentate complex with ferric iron,[13] and other hydroxamates have also been shown to target PHDs and activate the HIF pathway due to their iron-chelating properties.[14] Since the polycyclic lipophilic scaffold of triterpenoids seems remarkably versatile in terms of protein surface recognition,[5] it was conjectured that, by introducing a chelating element, the "dock and bind" strategy of bardoxolone methyl (**2**) may be replicated in another biological setting, combining shape affinity associated with the lipophilic scaffold with the specific binding properties of the chelating element. The hydroxamates of some triterpenoid acids have been described,[15,16] and there was therefore a clear rationale, in terms of both potential target and chemical feasibility, for the project.

RESULTS AND DISCUSSION

Five PCTTAs were selected as starting materials based on their multigram availability by isolation [ursolic acid (**4a**), betulinic acid (**5a**), maslinic acid (**6a**)], purchase [oleanolic acid (**3a**), glycyrrhetinic acids (**7a**)], or semisynthesis (the oleanolic/glycyrrhetinic acid hybrid **8a**).[17] Hydroxyamidation was achieved, after protection of the 3-hydroxy group as an acetate, by activation as chloride (**3a**−**6a**, **8a**) or mixed phosphonic anhydride (**7a**),[18] followed by reaction with hydroxylammonium chloride in pyridine and deprotection, eventually affording the hydroxamate alcohols **3b**−**8b** (Scheme 1). The effect of an additional hydroxylamine-derived functional group was evaluated in the 3-hydroxyimino-28-hydroxamates **3d**−**5d**

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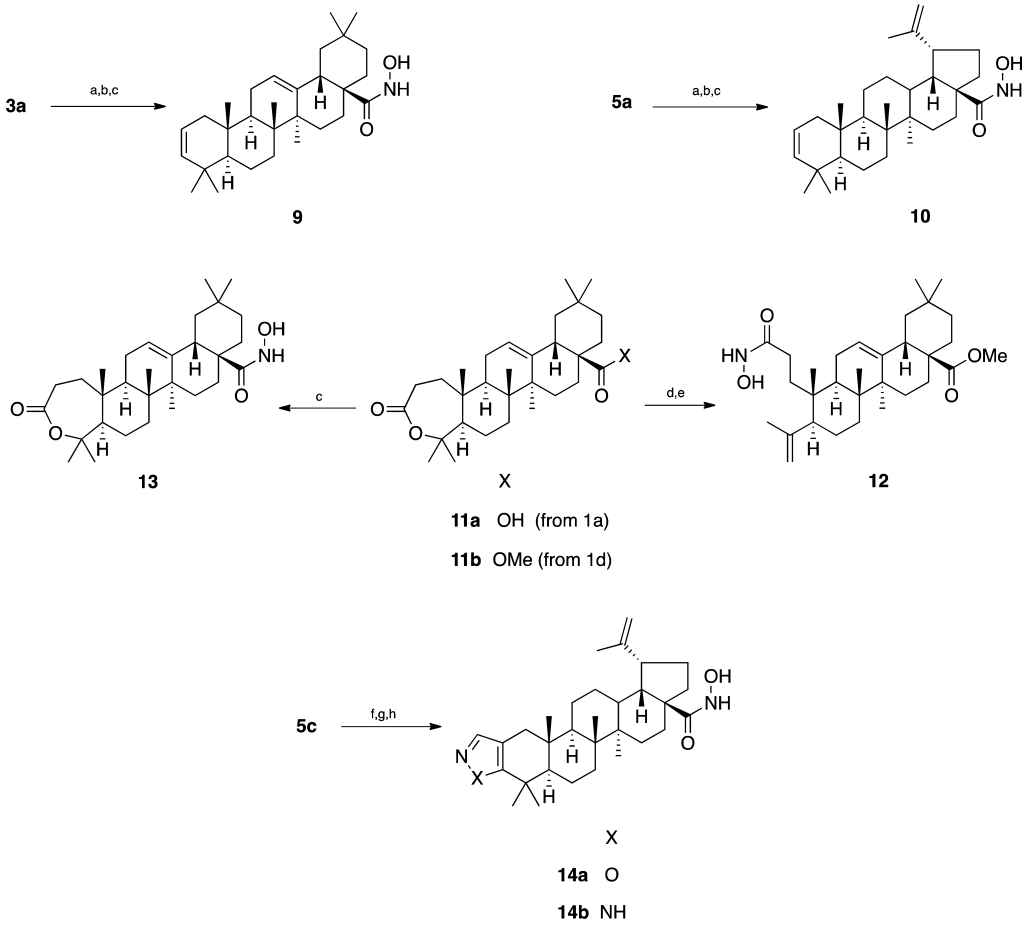
Scheme 2. Synthesis of Ring A-Modified PCTTA Hydroxamates[a]



[a](a) TsCl, pyridine; (b) NaOAc, DMF 120 °C (40−50 over two steps); (c) oxalyl chloride, CH_2Cl_2 40 °C then $NH_2OH{\cdot}HCl$, pyridine 40 °C (35−50%); (d) KOH, CH_2Cl_2/MeOH/H_2O, 3:3:1, (20%); (e) oxalyl chloride, CH_2Cl_2 40 °C then $NH_2OH{\cdot}HCl$, pyridine 40 °C (35%); (f) ethyl formate, NaOEt, toluene 50 °C for 19; (g) $NH_2OH{\cdot}HCl$, EtOH/H_2O, 80 °C for **14a** or NH_2NH_2, EtOH/H_2O 80 °C for **14b**; (h) oxalyl chloride, CH_2Cl_2 40 °C then $NH_2OH{\cdot}HCl$, pyridine 40 °C (40−50%). TsCl, *p*-toluenesulfonyl chloride; DMF, dimethylformamide.

obtained by 2-fold hydroxyamination of the 3-dehydro derivatives of oleanolic, ursolic, and betulinic acids (**3c**−**5c**, respectively).

Additional derivatives were obtained (Scheme 2) by modifications on ring A of oleanolic and betulinic acids, of which the hydroxamates (**3b** and **5b**, respectively) were found to show the most interesting bioactivity (vide infra). Thus, a double bond was generated on ring A by dehydration of the 3-hydroxy group, affording, after hydroxyamidation, the oleanane **9** and the lupane **10**. Alternatively, Baeyer−Villiger expansion of ring A of the 3-dehydro derivative of oleanolic acid (**3a**) or its methyl ester (**3e**) afforded respectively the lactones **11a** and **11b**. The carboxylate **11a** was next directly hydroxyamidated to **12**, while the lactonized methyl ester **11b** underwent acid-promoted β-elimination to an A-seco derivative and then was hydroxyamidated to **13**. Further changes on ring A were carried out on the 3-dehydro derivative of betulinic acid (**5c**), which, after formylation, was condensed with hydroxylamine or hydrazine to afford the heterocyclic 1,2-azoles **14a** and **14b**.

For comparison purposes, the hydroxamate group of the oleanolic (**3b**) and the betulinic (**5b**) hydroxamate lead was replaced by a series of different chelating elements [glycinamide, 2-hydroxybenzalglycinamide, (*N*-methyl-2-imidazolyl)hydroxymethyl], generating the analogues **15a**, **15b**, **16a**, **18a**, and **18b** (Scheme 3). These compounds were prepared from the starting acids by amidation (**15a** and **16a**) followed by *ortho*-hydroxybenzalization for the preparation of **15b** or from the corresponding C-28 aldehydes by addition of 2-lithio-*N*-methylimidazole (**18a** and **18b**).

HIF-1α transactivation assays were performed in NIH-3T3-EPO-Luc cells. The EPO-hypoxia response element (HRE)-luciferase reporter plasmid contains three copies of the HRE consensus sequence from the promoter of the erythropoietin gene fused to the luciferase gene and is therefore a useful surrogate marker for the screening of compounds for the induction of HIF-1α/HIF-2α stabilization.[19] None of the starting PCTTAs (**3a**−**8a**) showed hypoxia-mimetic activity, while their hydroxamates, with the notable exception of glycyrrhetinic acid (**7b**), were, to various extents, all significantly active. Conversely, no activity was observed in PCTTAs bearing at C-28 chelating groups different from the hydroxamate (**15a**, **15b**, **16a**, **19a**, and **19b**) or when the hydroxamate hydroxy group of **5b** was alkylated, as in **5e**. While emphasizing the relevance of the hydroxamate moiety and its chelating properties for activity, these observations also highlight the relevance of its location on the triterpenoid backbone, since the C-30 hydroxamate **7b** was inactive, while its corresponding C-28 hydroxamate **8b** was significantly active. Within the C-28 hydroxamates, activity was then fine-tuned by changes in connectivity and functionalization of the

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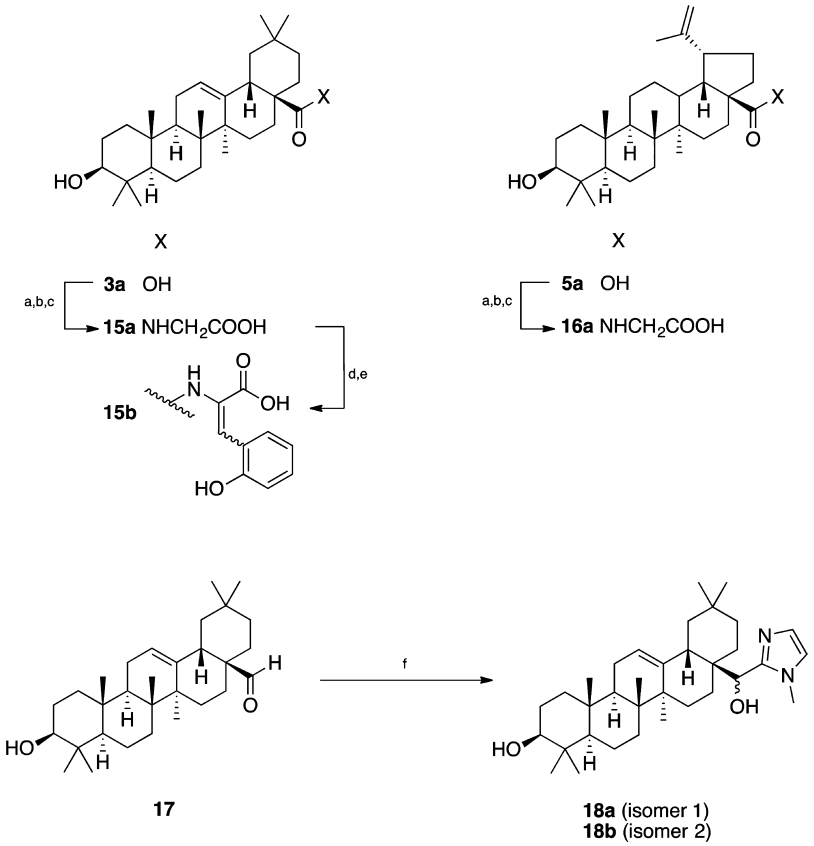
Scheme 3. Synthesis of PCTTAs Bearing at C-28 a Chelating Group[a]



[a](a) Ac_2O, DMAP, pyridine (quantitative); (b) oxalyl chloride, CH_2Cl_2 40 °C then glycine methyl ester hydrochloride, pyridine 40 °C; (c) NaOH 4 M, THF/MeOH, 1:1, 40 °C (50−60% over two steps); (d) salicylaldehyde, NaOAc, Ac_2O 140 °C; (e) NaOH 4 M, THF/MeOH, 1:1, 40 °C (38% over two steps); (f) 1-methylimidazole, *n*-BuLi, THF −78 °C (40% cumulative yield). Ac_2O, acetic anhydride; DMAP, 4-dimethylaminopyridine.

triterpenoid backbone. Thus, the lupane hydroxamate **5b** was more potent than its oleanane (**3b**, **6b**) and ursane (**4b**) analogues, while oxymation of the C-3 dehydro derivatives **3d** and **3e** was accompanied by a decrease of activity. Comparison of the activity of the hydroxamates of oleanolic (**3b**) and maslinic (**6b**) derivatives showed that introduction of a further hydroxy group on ring A was moderately beneficial, while fusion of ring A with a heterocyclic ring or dehydration of the 3-hydroxy group was not critical. Overall, changes on ring A did not significantly affect potency. On the other hand, its expansion in **12** was associated with a decrease of activity, while a certain activity was also observed in the A-*seco*-C-3 hydroxamate **13**.

Taken together, these observations show that, within the PCTTA scaffold, the presence of a hydroxamate group and its location are both critical for PHD targeting, since activity was observed when this group was located at C-28, but not at C-30, while no activity was observed when the C-28 hydroxamate was replaced by other chelating groups.

Next investigated was the biological translation of the acid-to-hydroxamate maneuver on other biological targets of the PCTTAs. These compounds are proved to be remarkably pleiotropic agents, targeting and/or modulating a series of transcription factors that include NF-κB (nuclear factor-kappa B), STAT3 (signal transducer and activator of transcription 3), Nrf2 [NFE2L2, or nuclear factor (erythroid-derived 2)-like 2], and the bile receptor TGR5.[5,19−22] Evaluation of the hydroxamates produced against these targets showed a detrimental effect on bioactivity, with only some residual Nrf2 and TGR5 activity found on the hydroxamate of ursolic acid and a complete loss of activity toward the NF-κB or STAT3 pathways (Table S1, Supporting Information). The carboxylate-to-hydroxamate transformation therefore has a dramatic modulating activity on the biological profile of PCTTAs, focusing their biological profile on the HIF pathway. Based on considerations of potency and target selectivity, the hydroxamate of betulinic acid (**5b**) was selected for further studies.

The effect of **5b** on the expression of HIF-1α at the protein level was investigated in 293T cells. This compound stabilized the HIF-1α protein without affecting the steady-state levels of the PHDs analyzed (Figure 1A). In kinetic experiments, its stabilizing activity on HIF-1α was detectable after 15 min, and the peak at 60 min was maintained after 3 h of stimulation (Figure 1B). Next, the induction of HIF-1α in cells cultured under hypoxic conditions was investigated in 293T cells, subjecting them to hypoxia (1% O_2) in the presence or absence of **5b**. The levels of HIF-1α stabilization were similar to those observed under hypoxia conditions, and the combination of both stimuli produced no significant change (Figure 1C). In accordance with these findings, the PHD2 levels were almost unaffected in response to both conditions (Figure 1C). These observations suggest that the hypoxia-mimetic activity of **5b** is associated with inhibition of prolyl

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Table 1. Effect of Triterpenoid Hydroxamates on HIF Pathway Activation

compound	efficacy HIF-1α (IRA coefficient)[a]	potency EC_{50} HIF-1α (μM)
1a−8a		(>50)
3b	0.39	16.4
3d	0.43	3.8
8b	0.15	11.4
9	0.16	5.0
12	1.19	5,8
13	2.2	7,5
5b	0.36	4.8
5d	0.31	6.8
10	0.48	3.2
14a	0.55	2.6
14b	0.34	2.4
4b	0.17	7.7
4d	0.1	8.9
6b	0.1	7.1
7b		(>50)

[a]EC_{50} and IRA (intrinsic relative activity) values were determined relative to 150 μM deferoxamine (DFX) using the following equation: IRA coefficient = $(EC_{50\text{-DFX}} \times E_{max})/(EC_{50} \times E_{max\text{-DFX}})$, where EC_{50} and E_{max} denote EC_{50} and E_{max} of the agonist, and $EC_{50\text{-DFX}}$ and $E_{max\text{-DFX}}$ denote EC_{50} and E_{max} values of the standard agonist DFX.

hydrolase functional activity, as further shown by a close correlation between the inhibition of HIF-1α hydroxylation and HIF-1α stabilization in cells preincubated with the proteasome inhibitor MG132 (Figure 1D).

The nature of the interaction with the HIF pathway was evaluated by assessing the induction of EPO-Luc activity in wash-out experiments where **5b** was removed from the cell culture by washing the cells with phosphate-buffered saline (PBS) after 1 h of treatment, and the EPO-Luc activity was then measured after a further 5 h. Activity was greatly reduced 5 h after removal of **5b** from the cell medium, as expected for a reversible inhibitor (Figure 1E). Remarkably, competition assays demonstrated that betulinic acid (**5a**) (50 μM) inhibited the hypoxia-mimetic activity of its hydroxamate derivative **5b** (Figure 1F), suggesting a "dock and bind" mechanism, where the triterpenoid scaffold is involved in target recognition, presumably by shape complementarity associated with its lipophilic core, and the hydroxamate reinforces it with polar interactions and/or chelation. This was supported by the observation that O-alkylation of the hydroxamate function of **5b** completely abolished activity (compound **5e**).

Many compounds have shown promise for HIF-targeted therapies, but HIF-1-associated neurotoxicity and/or cytotoxicity have, in most cases, hindered their advancement to in vivo studies, with a resulting shortage of preclinically validated leads.[11] Provided herein is evidence that pentacyclic triterpenoid acid hydroxamates are a class of novel and selective modulators of HIF-1 signaling. Their mechanism of activity goes beyond generic chelating properties, since replacement of the carboxylate with other chelating elements was not associated with activity, while a definite spatial relationship between the hydroxamate moiety and the triterpenoid core was critical for their observed privileged effects.

EXPERIMENTAL SECTION

General Experimental Procedures. Commercially available reagents and solvents were purchased from Aldrich or Alfa-Aesar and were used without further purification. *N,N′*-Dimethylformamide (DMF) was dried over a neutral alumina pad and stored on 4 Å activated molecular sieves. Dichloromethane was dried by distillation from P_2O_5 and stored on 4 Å activated molecular sieves. Pyridine was dried over a neutral alumina pad and stored on activated 4 Å molecular sieves under nitrogen. When needed, the reactions were performed in flame- or oven-dried glassware under a positive pressure of dry nitrogen. For spectroscopic characterization, a JEOL ECP 300 MHz spectrometer was used for ^1H and ^{13}C spectra. Chemical shifts are reported in parts per million (ppm) using the residual solvent peak as reference ($CHCl_3$ at δ 7.27). A Thermo Finningan LCQ-deca XP-Plus equipped with an ESI source and an ion trap detector was employed for mass spectrometry. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230−400 mesh ASTM). Thin-layer chromatography (TLC) was carried out on 5 × 20 cm plates with a layer thickness of 0.25 mm (Merck silica gel 60 F254). When necessary, $KMnO_4$ was used for visualization. Compounds **3b**,[16] **4b**,[16] **5b**,[15] **5e**,[15] **7b**,[18] **11a**,[23] and **11b**,[23] as well as **15a**,[24] **16a**,[25] and **17**[26] have previously been reported.

Synthesis of Triterpenoid Hydroxamates. *Protocol A (via Chlorides).* Synthesis of **5b** as an example: (a) Acetylation: To an ice-cold solution of betulinic acid **5a** (1 g, 2.19 mmol, 1 equiv/mol) in dry pyridine (10 mL) were added sequentially acetic anhydride (413 μL, 4.38 mmol, 2 equiv/mol) and DMAP (27 mg, 0.1 equiv/mol). The reaction was stirred at room temperature for 3 h, quenched with methanol, diluted with 2 N H_2SO_4, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure to give crude acetylbetulinic acid, used for the next step without further purification. (b) Hydroxyamidation: To an ice-cold solution of crude acetylbetulinic acid (1.09 g, 2.19 mmol, 1 equiv/mol) in dry dichloromethane (15 mL) was added dropwise oxalyl chloride (1.13 mL, 13.14 mmol, 6 equiv/mol), and the mixture was heated at 40 °C for 1.5 h. The solvent was then removed at reduced pressure, the residue dissolved in dry pyridine, and hydroxylammonium chloride (913 mg, 13.14 mmol, 6 equiv/mol) added. The reaction was heated at 40 °C for 3 h, quenched with 2 N H_2SO_4, and extracted with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure. The crude reaction product was further purified over silica gel (petroleum ether/EtOAc, 7:3) to afford 667 mg (60%) of residue. (c) Deprotection: To a solution of acetylbetulinyl hydroxamate (667 mg, 1.31 mmol) in THF/MeOH, 1:1 (8 mL), was added 4 N NaOH (16.4 mL, 65.5 mmol; 50 molar equiv). The mixture was heated at 40 °C overnight, quenched with 2 N H_2SO_4, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated. The crude product was purified over silica gel (petroleum ether/EtOAc, 5:5), affording 330 mg (55%) of **5b** as an off-white powder. For physical and spectroscopic data, see ref 15. The data for the new compounds are as follows.

*(2α,3β)-2,3-Dihydroxy-N-hydroxyolean-12-en-28-amide (**6b**):* off-white solid (45%); IR (KBr) 2939, 2867, 1662, 1450, 1031, 883 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.44 (1H, brt), 3.72−3.61 (1H, m), 2.99 (1H, d, J = 9.5 Hz), 2.45 (1H, d, J = 12.2 Hz), 1.15 (3H, s), 1.02 (3H, s), 0.98 (3H, s), 0.90 (3H, s), 0.87 (3H, s), 0.82 (3H, s), 0.78 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.7, 144.9, 124.0, 78.5, 76.6, 55.1, 48.0, 46.2, 46.2, 45.5, 42.1, 40.7, 39.5, 38.1, 36.7, 33.9, 33.0, 32.1, 32.0, 30.7, 29.7, 27.1, 25.9, 23.7, 23.5, 22.8, 18.1, 17.3, 16.7, 16.3; ESIMS m/z 488 $(M + H)^+$.

*(3β)-3-Hydroxy-11-oxo-olean-12-ene-N-hydroxy-28-amide (**8b**):* pale yellow solid (38%); IR (KBr) 2944, 2865, 1651, 1464, 1209, 1039, 994, 733 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.67 (1H, s), 3.20 (1H, t, J = 6.1 Hz), 2.74 (2H, d, J = 12.2 Hz), 2.32 (1H, s), 2.10−2.02 (1H, m), 1.18 (3H, s), 0.96 (3H, s), 0.94 (3H, s), 0.90 (9H, s), 0.77 (3H, s) (only readily obsesrved peaks are reported); ^{13}C

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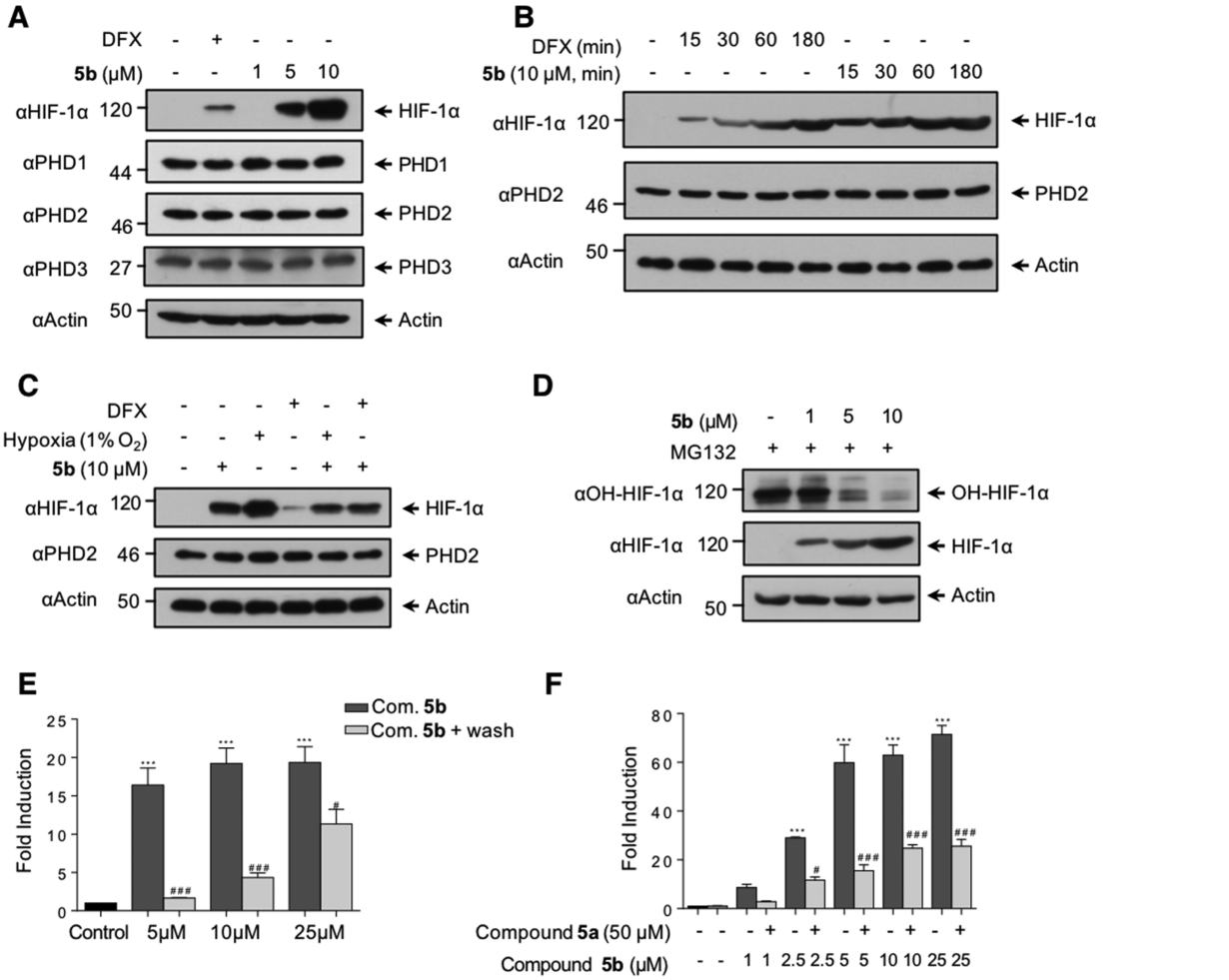

Figure 1. Compound **5b** mediates HIF-1α stabilization. (A) HEK-293T cells were stimulated with compound **5b** at the indicated concentrations of DFX (100 μM) for 3 h, and the expression of HIF-1α, PHD1, PHD2, and PHD3 was analyzed by Western blots. (B) Time-course induction of HIF-1α stabilization. (C) Effect of **5b** in the presence or the absence of low oxygen (1% O_2) on HIF-1α stabilization. (D) HEK-293T cells were treated with **5b** for 6 h in the presence of MG132, and the levels of hydroxylated HIF-1α and HIF-1α were determined by immunoblot. (E) NIH-3T3-EPO-Luc cells were pretreated with **5b** for 1 h and then washed or not with PBS and incubated in complete medium for 6 h. $^{***}p < 0.001$ for compound **5b**-treated cells (no wash) vs untreated cells; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ for **5b**-treated cells (wash vs no wash cells) (one-way ANOVA followed by Tukey's test). (F) NIH-3T3-EPO-Luc cells were preincubated with **5a** (betulinic acid) (50 μM) for 30 min and then treated with **5b** at the indicated concentrations for 6 h. Luciferase activity was measured in the cell lysates, and fold induction relative to untreated cells is shown. $^{***}p < 0.001$ for **5b**-treated cells vs untreated cells; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ for **5b**-treated cells (wash) vs **5b** + **5a**-treated cells (one-way ANOVA followed by Tukey's test). Data represent the means ± SD (n = 3).

NMR (75 MHz, $CDCl_3$) δ 200.2, 175.8, 167.9, 128.0, 78.8, 62.1, 55.0, 45.2, 44.7, 43.6, 40.9, 39.2, 37.3, 33.7, 32.8, 32.7, 32.1, 30.7, 29.7, 28.1, 27.4, 27.3, 23.7, 23.4, 23.3, 19.0, 17.5, 16.2, 15.6, 14.2; ESIMS m/z 486 $(M + H)^+$.

Protocol B (via Mixed Phosphoric Anhydride). Synthesis of **7b**: To a stirred solution of a PPAA (50% in EtOAc, 146 μL, 0.264 mmol, 1.2 molar equiv) in acetonitrile (3 mL) were sequentially added triethylamine (126 μL, 0.88 mmol, 4 molar equiv) and glycyrrhetic acid (100 mg, 0.22 mmol, 1 molar equiv). After stirring 30 min at room temperature, hydroxylammonium chloride (31 mg, 0.44 mmol, 2 molar equiv) was added, and stirring was continued overnight at room temperature. The reaction was then worked up by dilution with EtOAc and washing with brine. The organic phases were dried over Na_2SO_4 and evaporated, and the residue was purified over silica gel (petroleum ether/EtOAc, 5:5), affording 69 mg (64%) of **7b**[18] as a white powder.

Triterpenoid Oxyminohydroxamates. Synthesis of **3d** an example: (a) To a stirred solution of oleanolic acid (**3a**, 500 mg, 1.1 mmol) in EtOAc/acetone, 1:1 (7 mL) was added dropwise Jones reagent until the reaction turned green. The mixture was diluted with brine and extracted with EtOAc, and the combined organic phases were dried over Na_2SO_4 and evaporated. The residue was purified by silica gel (petroleum ether/EtOAc, 8:2), affording 474 mg (95%) of dehydrooleanolic acid.[27] (b) Oxyminohydroxylation: To an ice-cold solution of dehydrooleanolic acid (474 mg, 1.04 mmol, 1 molar equiv) in dry dichloromethane (7 mL) was added dropwise oxalyl chloride (353 μL, 6.24 mmol, 6 molar equiv), and the mixture was heated at 40 °C for 1.5 h. The solvent was then removed at reduced pressure, the residue dissolved in dry pyridine, and hydroxylammonium chloride (430 mg, 6.24 mmol, 6 molar equiv) added. The reaction was heated at 40 °C for 3 h, quenched with 2 N H_2SO_4, and extracted with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure. The crude reaction product was further purified over silica gel (petroleum ether/EtOAc, 5:5) to afford 353 mg (70%) of **3d** as an off-white solid: IR (KBr) 2944, 2859, 1701, 1674, 1632, 1463, 1389, 1364, 924 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.43 (1H, brt), 3.07 (1H, bdt, J = 14.9), 2.46 (1H, m), 1.98 (3H, m), 1.13 (10H, s), 1.07 (6H, s), 1.05 (3H, s), 0.86 (6H, s), 0.82 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.7, 167.5, 144.9, 123.8, 55.7, 47.1, 46.2, 45.5, 42.0, 40.8, 40.3, 39.4, 38.4,

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37.0, 33.9, 32.9, 31.9, 30.7, 29.7, 29.2, 27.2, 25.7, 25.5, 23.7, 23.5, 23.4, 19.0, 17.3, 16.7, 14.9; ESIMS m/z 485 $(M + H)^+$.

*3-Hydroxyimino-N-hydroxyurs-12-en-28-amide (**4d**):* pale yellow solid (70%); IR (KBr) 2956, 2833, 1729, 1630, 1423, 1390, 1374, 982, 949 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.40 (1H, brt), 3.07 (1H, brt, J = 15.6), 2.12 (1H, m), 1.24 (3H, s), 1.15 (3H, s), 1.08 (3H, s), 1.06 (3H, s), 1.03 (3H, s), 0.94 (3H, s), 0.81 (3H, s) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.3, 167.7, 140.6, 126.5, 55.7, 52.1, 47.0, 42.5, 40.2, 39.6, 39.4, 39.0, 38.5, 37.0, 36.7, 32.2, 30.6, 29.7, 27.7, 27.4, 24.8, 23.5, 23.4, 23.3, 21.1, 19.0, 17.3, 17.2, 16.8, 15.1; ESIMS m/z 485 $(M + H)^+$.

*3-Hydroxyimino-N-hydroxylup-20(29)-en-28-amide (**5d**):* off-white solid (65%); IR (KBr) 2966, 2831, 1751, 1715, 1665, 1453, 1449, 1034, 1007, 984, 866 cm^{-1}; ^1H NMR (300 MHz, $CO(CD_3)_2$) δ 4.73 (1H, s), 4.61 (1H, d, J = 6.1 Hz), 3.04−3.00 (2H, m), 2.32 (1H, s), 1.68 (3H, s), 1.24 (6H, s), 1.22 (3H, s), 1.12 (3H, s), 0.97 (3H, s), 0.96 (3H, s), 0.91 (3H, s) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CO(CD_3)_2$) δ 176.7, 164.1, 150.5, 109.6, 56.0, 55.5, 55.3, 50.2, 47.1, 42.5, 40.8, 40.2, 38.7, 38.2, 37.9, 37.2, 34.0, 33.3, 30.8, 29.4, 27.4, 25.6, 22.9, 21.5, 21.2, 19.4, 19.1, 16.1, 15.8, 14.6; ESIMS m/z 485 $(M + H)^+$.

Synthesis of Δ^2-Hydroxamates. Synthesis of **9** as an example: To a stirred solution of oleanolic acid (**3a**, 500 mg, 1.1 mmol, 1 molar equiv) in dry pyridine (9 mL) was added *p*-toluenesulfonyl chloride (735 mg, 3.8 mmol, 3.8 molar equiv). The solution was stirred at room temperature for 24 h under a nitrogen atmosphere, diluted with water, and then extracted with CH_2Cl_2. The organic phases were washed with saturated $KHSO_4$ solution, dried over Na_2SO_4, and concentrated at reduced pressure. The crude product was diluted in DMF (6 mL), sodium acetate (315 mg, 2.3 mmol) was added, and the mixture was heated at 120 °C for 24 h under a nitrogen atmosphere. The mixture was diluted with brine and extracted with CH_2Cl_2. The organic phases were dried over Na_2SO_4 and evaporated. The residue was purified over silica gel (petroleum ether/EtOAc, 9:1), affording 221 mg (42%) of Δ^2-oleanolic acid,[27] diluted in dry CH_2Cl_2 (4 mL), and cooled at 0 °C. Oxalyl chloride (249 μL, 2.9 mmol, 6 molar equiv) was then added dropwise, and the mixture was heated at 40 °C for 1.5 h. The solvent was then removed at reduced pressure, the residue dissolved in dry pyridine, and hydroxylammonium chloride (201 mg, 2.9 mmol, 6 molar equiv) added. The reaction was heated at 40 °C for 3 h, quenched with 2 N H_2SO_4, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure. The crude reaction product was purified over silica gel (petroleum ether/EtOAc, 7:3), affording 104 mg (48%) of **9** as a yellowish oil: IR (KBr) 2949, 2868, 1632, 1461, 1387, 1362, 910, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.43−5.32 (3H, m), 2.44 (1H, d, J = 11.3 Hz), 1.14 (3H, s), 0,97 (6H, s), 0.87 (12H, s) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4, 144.5, 138.0, 124.1, 121.3, 51.9, 46.4, 46.1, 45.5, 42.1, 41.0, 40.7, 39.5, 36.1, 34.5, 34.0, 33.0, 31.9, 31.8, 31.6, 30.7, 27.2, 25.9, 25.7, 23.8, 23.5, 22.9, 19.6, 16.3, 15.6; ESIMS m/z 454 $(M + H)^+$.

*Lupa-2,20(29)-diene-N-hydroxy-28-amide (**10**):* yellowish powder (54%); IR (KBr) 2936, 2868, 1717, 1643, 1448, 1374, 881, 731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.38−5.28 (2H, m), 4.69 (1H, s), 4.56 (1H, s), 3.01 (1H, t, J = 10.7 Hz), 2.37 (1H, t, J = 12.1 Hz), 1.63 (3H, s), 1.21 (3H, s), 0.93 (3H, s), 0,89 (3H, s), 0.82 (3H, s), 0.81 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 150.5, 137.9, 121.6, 109.65, 54.3, 52.1, 50.4, 49.2, 42.3, 40.8, 38.4, 37.9, 36.4, 34.6, 33.5, 32.8, 31.7, 30.9, 30.8, 29.7, 29.3, 25.6, 22.6, 19.5, 16.4, 15.8, 14.6, 14.5, 14.3; ESIMS m/z 454 $(M + H)^+$.

Triterpenoid Hydroxamates from the Products of Bayer−Villiger Fragmentation (11a, 11b). *Olean-12-ene-N-hydroxy-28-amid-3-oic acid ε-lactone (**13**):* prepared from **11a**[23] according to the hydroxyamidation protocol A. White solid (35%); ^1H NMR (300 MHz, $CDCl_3$) δ 5.45 (1H, brt), 2.63 (2H, t, J = 5.4 Hz), 2.47 (1H, brdd, J = 9.4 Hz), 2.05 (2H, m), 1.52 (3H, s), 1.46 (3H, s), 1.29 (3H, s), 1.16 (3H, s), 0.90 (3H, s), 0.88 (6H, s), 0.83 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 175.4, 144.6, 123.6, 86.4, 55.2, 47.2, 46.1, 45.5, 42.2, 40.8, 40.0, 39.4, 37.4, 33.9, 32.96, 32.91, 31.99, 31.92, 30.7, 27.1, 25.5, 25.4, 23.9, 23.6, 23.4, 22.6, 20.8, 16.5, 16.4; ESIMS m/z 486 $(M + H)^+$.

*A-seco-4,12-dienolean-N-hydroxy-3-amide-28-methyl ester (**12**):* prepared from **11b**[23] after KOH hydrolysis and hydroxyamidation according to protocol A. White solid (20%): ^1H NMR (300 MHz, $CDCl_3$) δ 5.29 (1H, brt), 4.85 (1H, s), 4.66 (1H, s), 3.62 (3H, s), 2.74 (1H, brdd, J = 9.7 Hz), 1.71 (3H, s), 1.10 (3H, s), 0.90 (6H, s), 0.87 (6H, s), 0.74 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 171.9, 147.5, 143.7, 122.2, 113.7, 60.54, 51.6, 50.8, 46.8, 45.8, 42.2, 41.4, 39.3, 39.1, 38.1, 33.9, 33.1, 32.4, 31.4, 30.7, 27.7, 25.8, 24.4, 23.6, 21.1, 20.8, 19.4, 18.4, 16.9, 14.2; ESIMS m/z 500 $(M + H)^+$.

Heterocyclic Hydroxamates. Synthesis of **14a** as an example: (a) Formylation: To a solution of **5c** (300 mg, 0.65 mmol) in toluene (70 mL) were sequentially added NaOEt (221 mg, 3.25 mmol, 5 molar equiv) and ethyl formate (241 mg, 3.25 mmol, 5 molar equiv). The reaction mixture was stirred at 50 °C overnight, quenched with 2 N H_2SO_4, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure to afford 2-formyl-3-oxobetulinic acid[28] as a colorless oil, which was used without further purification for the next step. (b) 1,2-Diazole formation (reaction with hydroxylammonium chloride as an example): To a stirred solution of crude 2-formyl-3-oxo-betulinic acid (310 mg, 0.64 mmol, 1 molar equiv) in ethanol/H_2O, 9:1 (6 mL), was added hydroxylammonium chloride (400 mg, 5.76 mmol, 9 molar equiv). The reaction mixture was heated at 80 °C for 5 h, diluted with H_2O, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure, affording the crude product as a brown oil, used without further purification. (c) Hydroxyamidation: The reaction was carried out according to protocol A, affording compound **14a** (41% from **5c**) as a white powder.

*Lup-2-eno[2,3-d]-isoxazol-N-hydroxy-28-amide (**14a**):* white powder; IR (KBr) 2953, 2867, 1714, 1632, 1508, 1455, 1367, 956, 887 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 10.37 (1H, s, NH), 8.42 (1H, s, OH), 8.26 (1H, s), 4.67 (1H, s), 4.55 (1H, s), 3.00 (1H, t, J = 9.3 Hz), 2.61 (1H, t, J = 12.0 Hz), 1.64 (3H, s), 1.40 (3H, s), 1.22 (3H, s), 1.11 (3H, s), 0.93 (3H, s), 0.74 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 151.3, 151.0, 109.9, 109.4, 54.0, 53.3, 50.6, 49.0, 48.9, 46.7, 42.4, 38.9, 37.3, 35.6, 34.8, 33.4, 32.6, 30.9, 29.0, 25.7, 21.7, 19.5, 18.7, 16.4, 16.2, 14.8; ESIMS m/z 495 $(M + H)^+$.

*1′H-Lup-20(29)-eno[3,2-c]-pyrazol-N-hydroxy-28-amide (**14b**):* yellowish powder (overall 48% from **5a**); IR (KBr) 2986, 2798, 1700, 1655, 1508, 1390, 1287, 1035, 851, 739 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2CO$) δ 7.17 (1H, s), 4.72 (1H, s), 4.58 (1H, s), 3.17 (1H, s), 2.65 (1H, s), 1.69 (3H, s), 1.28 (3H, s), 1.18 (3H, s), 1.02 (3H, s), 0.98 (3H, s), 0.80 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 172.4, 151.0, 149.1, 132.8, 111.9, 109.0, 59.7, 50.0, 53.7, 50.5, 49.2, 46.8, 42.2, 40.7, 38.6, 37.9, 37.7, 36.6, 33.5, 33.4, 32.3, 30.8, 30.6, 25.7, 23.3, 21.4, 19.1, 18.7, 15.6, 14.2, 13.7; ESIMS m/z 494 $(M + H)^+$.

*2-(28-Oleanoylamido)-3-(2-hydroxyphenyl)acrylic acid (**15b**).* In a sealed tube, a solution of oleanoylglycineamide (**15a**,[24] 200 mg, 0.39 mmol) in acetic anhydride (92 μL, 0.97 mmol, 2.5 molar equiv) was treated with salicylic aldehyde (72 mg, 0.58 mmol, 1.5 molar equiv) and NaOAc (24 mg, 0.29 mmol, 0.75 molar equiv). The reaction was heated at 140 °C for 1.5 h, then diluted with MeOH, EtOAc, and brine. The organic phases were dried over Na_2SO_4 and evaporated at reduced pressure. The crude azalactone (154 mg, 0.26 mmol) was dissolved in THF/MeOH, 1:1 (4 mL), and 4 N NaOH (5 mL, 20 mmol; 51 molar equiv). The mixture was heated at 40 °C overnight, quenched with 2 N H_2SO_4, and extracted with EtOAc. The organic phases were washed with brine, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product was purified over silica gel (petroleum ether/EtOAc, 9:1, as eluant), affording 82 mg (35% from **15a**) of **15b** as a yellowish solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.76 (1H, s), 8.70 (1H, s), 7.35 (3H, m), 5.63 (1H, brt), 3.18 (1H, dd, J = 7.9, 1.5 Hz), 2.73 (1H, dd, J = 11.4, 0.9 Hz), 1.18 (3H, s), 0.95 (3H,

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s), 0.93 (6H, s), 0.85 (3H, s), 0.73 (3H, s), 0.63 (3H, s) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 158.9, 149.9, 142.8, 129.4, 127.8, 125.1, 124.8, 124.2, 122.9, 120.1, 116.3, 79.0, 55.1, 47.9, 47.6, 46.5, 42.3, 41.9, 39.4, 38.8, 38.6, 36.9, 34.2, 33.0, 32.6, 32.4, 30.8, 28.1, 27.5, 27.2, 25.9, 24.2, 23.6, 18.3, 16.4, 15.6, 15.4; ESIMS m/z 618 $(M + H)^+$.

*N-Imidazolyl Semiaminals **18a** and **18b***. To a stirred and cooled (−78 °C) solution of methyl imidazole (75 mg, 0.91 mmol, 2 molar equiv) in dry THF was added dropwise *n*-BuLi (0.8 M, 1.13 mL, 0.908 mmol, 2 molar equiv). After 15 min a solution of **17** (200 mg, 0.454 mmol, 1 molar equiv) in dry THF (3 mL) was added slowly, and the reaction was stirred overnight at room temperature. The reaction was quenched with H_2O and washed with EtOAc, and the combined organic phases were dried over Na_2SO_4 and evaporated at reduced pressure. The crude product was purified over silica gel (petroleum ether/EtOAc, 9:1, as eluant), affording **18a** (35 mg, 15%) and **18b** (58 mg, 25%). **18a**: IR (KBr) 3112, 2945, 2872, 1463, 1365, 1274, 1000, 936, 734 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (1H, s), 6.78 (1H, s), 5.36 (1H, t, J = 3.3 Hz), 4.85 (1H, s), 3.71 (3H, s), 3.20 (1H, m), 2.73 (1H, dd, J_1 = 3.9 Hz, J = 9.1 Hz), 1.19 (3H, s), 1.03 (3H, s), 0.98 (3H, s), 0.93 (6H, s), 0.88 (6H, s), 0.77 (3H, s) (only readily peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.3, 144.2, 126.8, 123.1, 121.6, 79.0, 68.8, 55.2, 47.6, 46.9, 41.7, 41.6, 41.0, 40.0, 38.8, 38.6, 37.0, 34.2, 33.9, 33.2, 32.5, 30.8, 28.1, 27.2, 26.3, 25.6, 25.4, 23.7, 23.4, 22.7, 18.3, 17.4, 15.6, 15.6; ESIMS m/z 523 $(M + H)^+$. **18b**: IR (KBr) 3100, 2967, 2855, 1459, 1405, 1137, 1079, 1035, 920, 765 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (1H, s), 6.79 (1H, s), 5.18 (1H, t, J = 3.3 Hz), 4.71 (1H, s), 3.66 (3H, s), 3.21 (1H, m), 2.12 (1H, dt, J = 14.4 and 5.1 Hz), 1.20 (3H, s), 1.01 (3H, s), 0.99 (3H, s), 0.92 (3H, s), 0.80 (3H, s), 0.79 (3H, s), 0.50 (3H, s) (only readily observed peaks are reported); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2, 145.5, 127.0, 124.0, 121.0, 78.9, 68.4, 55.1, 47.7, 47.5, 42.0, 41.7, 39.9, 38.8, 38.5, 37.0, 34.2, 34.0, 32.9, 32.3, 30.7, 28.1, 27.2, 26.6, 25.7, 25.1, 23.7, 23.4, 23.0, 18.4, 17.1, 15.6, 15.4; ESIMS m/z 523 $(M + H)^+$.

Cell Lines and Reagents. HEK-293T cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 1% (v/v) penicillin/streptomycin. Human brain microvascular endothelial cells (HBMECs) were maintained in endothelial cell medium (ScienCell, San Diego, CA, USA) supplemented with 5% fetal bovine serum, 1% endothelial cell growth supplement (ECGS), and 1% penicillin/streptomycin. The mouse NIH3T3-EPO-luc cells were stably transfected with the plasmid Epo-Luc plasmid. The EPO-HRE-luciferase reporter plasmid contained three copies of the HRE consensus sequence from the promoter of the erythropoietin gene fused to the luciferase gene and was therefore a useful surrogate marker for screening compounds for the induction of HIF-1α/HIF-2α stabilization and activation. The generation and characterization of the NIH-KBF-Luc, Hela-STAT-Luc, HaCaT-ARE-Luc, and CHO-TGR5-CRE-Luc cells have been described elsewhere.[6]

Luciferase Assays. For determining anti-NF-κB activities NIH-3T3-KBF-Luc cells were stimulated with TNFα (20 ng/mL) in the presence or the absence of the compounds for 6 h. For the activation of the antioxidant response element (ARE), Nrf2 HaCaT-ARE-Luc cells were stimulated with the compounds for 6 h. For anti-STAT3 activities, HeLa-STAT-3-Luc cells were stimulated with γIFN (20 IU/mL) in the presence or the absence of each test compound for 6 h. For activation of the TGR5 receptor, the cells were stimulated with the compounds for 6 h. For the activation of the HIF pathway, NIH-3T3-EPO-Luc cells were stimulated with the test compounds for 6 h. After treatment, the cells were washed twice in phosphate-buffered saline and lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM dithiothreitol, 1% Triton X-100, and 7% glycerol for 15 min at room temperature in a horizontal shaker. After centrifugation, luciferase activity in the supernatant was measured using a GloMax 96 microplate luminometer (Promega, Madison, WI, USA) following the instructions provided in the luciferase assay kit (Promega).

Western Blots. After treatment, the cells were washed with PBS and the proteins were extracted in 50 μL of lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10% glycerol, and 1% NP-40) supplemented with 10 mM NaF, 1 mM Na_3VO_4, 10 μg/mL leupeptine, 1 μg/mL pepstatin and aprotinin, and 1 μL/mL saturated phenylmethane sulfonyl fluoride (PMSF). A 30 μg amount of proteins was boiled at 95 °C in Laemmli buffer and electrophoresed in 10% SDS/PAGE gels. Separated proteins were transferred to PVDF membranes (20 V for 30 min) and blocked in tris-buffered saline (TBS) solution containing 0.1% Tween 20 and 5% nonfat dry milk for 1 h at room temperature. Immunodetection of specific proteins was carried out by incubation with primary antibody against HIF-1α (1:1000; BD Biosciences, #610959, San Jose, CA, USA), PHD1 (1:1000; Abcam, Cambridge, UK), PHD2 (1:1000; Abcam), PHD3 (1:1000; Abcam), OH-HIF-1α (1:1000; Cell Signaling, Danvers, MA, USA), and β-actin (1:10.000; Sigma) overnight at 4 °C. After washing the membranes, horseradish peroxidase-conjugated secondary antibody was added and detected by a chemiluminescence system (GE Healthcare Europe GmbH).

Statistical Analysis. All the in vitro data are expressed as means ± SD. One-way ANOVA followed by Tukey's or Dunnett's post hoc test was used to determine the statistical significance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00514.

Table S1 (activity against transcription factors) and original spectroscopic data for the novel compounds (PDF)

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Notes

The authors declare no competing financial interest.

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