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Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation

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Author contributions: T.V., A.C.-K., W.H., and O.W. contributed equally to this work. A.C.-K., O.W., T.K., D.I., P.I., and M.S. contributed to medicinal chemistry experiments. W.H., X.B., L.P., and I.B. designed, analyzed, and performed animal and cell culture experiments. T.V., A.C.-K., W.H., O.M., B.M.F.H., S.K., C.v.N., C.B.-B., C.K., M.A., I.B., and T.H. designed, performed, and analyzed cell biology experiments. T.V., A.C.-K., L.P., X.B., O.L., A.-S.J., A.J.J., E.W., E.J.H., C.B.J.P., M.G., and T.H. designed, performed, and analyzed biochemical and high-throughput experiments. G.M. and P.S. designed, performed, and analyzed the structural biology experiment. T.V., A.S., and H.E.K. designed, performed, and analyzed LC-MS/MS experiments. A.C.-K., A.M., J.A.-W., and R.A.Z. designed, performed, and analyzed hydrogendeuterium exchange experiments. P.B., P.A., A.C.-K., C.G., K.S., T.P., U.W.B., and A.R. designed, performed, and analyzed ADME, pharmacology, and toxicology experiments. T.V., A.C.-K., I.B., and T.H. wrote the manuscript. All authors discussed results and approved the manuscript.

Competing interests: T.V., A.C.-K., O.W., T.K., and T.H. are listed as inventors on a provisional U.S. patent application no. 62/636983, covering OGG1 inhibitors. The patent is fully owned by a nonprofit public foundation, the Helleday Foundation, and T.H. and U.W.B. are members of the foundation board developing OGG1 inhibitors toward the clinic. An inventor reward scheme is under discussion. The remaining authors declare no competing financial interests.

Data and materials availability: Mouse inflammatory cytokines and receptors PCR array data have been deposited in the Gene Expression Omnibus (GEO), NCBI, and is accessible through GEO series accession nos. GSE106785 and GSE116809. The atomic coordinates and structure factors (codes 6G3X and 6G3Y) have been deposited in the Protein Data Bank (www.rcsb.org). The supplementary materials section contains additional data. All other data needed to evaluate the conclusions in this paper are present in either the main text or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Abstract

The onset of inflammation is associated with reactive oxygen species and oxidative damage to macromolecules like 7,8-dihydro-8-oxoguanine (8-oxoG) in DNA. Because 8-oxoguanine DNA glycosylase 1 (OGG1) binds 8-oxoG and because *Ogg1*-deficient mice are resistant to acute and systemic inflammation, we hypothesized that OGG1 inhibition may represent a strategy for the prevention and treatment of inflammation. We developed TH5487, a selective active-site inhibitor of OGG1, which hampers OGG1 binding to and repair of 8-oxoG and which is well tolerated by mice. TH5487 prevents tumor necrosis factor- α -induced OGG1-DNA interactions at guanine-rich promoters of proinflammatory genes. This, in turn, decreases DNA occupancy of nuclear factor κ B and proinflammatory gene expression, resulting in decreased immune cell recruitment to mouse lungs. Thus, we present a proof of concept that targeting oxidative DNA repair can alleviate inflammatory conditions in vivo.

Upon exposure to proinflammatory agents, cells produce increased levels of reactive oxygen species (ROS), which induce oxidative DNA damage. Guanine is particularly vulnerable because it has the lowest oxidation potential among canonical DNA bases (1,2), resulting primarily in 7,8-dihydro-8-oxoguanine (8-oxoG), particularly at guanine-rich promoter regions (3, 4). 8-Oxoguanine DNA glycosylase 1 (OGG1) binds with high affinity to 8-oxoG in double-stranded DNA to initiate DNA base excision repair. In addition to this role, OGG1 has distinct signal transduction functions (5–7), interacts with 8-oxoG in gene regulatory regions, and facilitates gene expression (3,7–12). These observations provide a potential explanation for the decreased inflammatory responses in *Ogg1*-deficient (*Ogg1*^{−/−}) mice (13–16), which are otherwise viable and largely healthy (17). Thus, we hypothesized that

small-molecule OGG1 inhibitors maybe clinically useful for the alleviation of inflammatory processes while still being well tolerated.

To screen for OGG1 inhibitors, we used a duplex oligonucleotide with the OGG1 substrate 8-oxo-7,8-dihydro-2'-deoxyadenosine and an excess of apurinic or apyrimidinic (AP) endonuclease 1 (APE1), which acts downstream of OGG1 and increases its turnover on damaged DNA (18) (Fig. 1, A and B). We screened a library containing 17,940 (table S1) and identified a hit molecule with a median inhibitory concentration (IC₅₀) of 8.6 μ M. During hit expansion, we developed TH5487 as a potent OGG1 inhibitor with an IC₅₀ of 342 nM, whereas structurally similar analogs TH2840 and TH5411 were inactive, with IC₅₀ values exceeding 100 μ M (Fig. 1, C and D, and figs. S1 to S3). This compound series was selective for OGG1; did not affect the activity of other DNA glycosylases (fig. S5A and table S2) or various Nudix hydrolases and diphosphatases (table S3); and did not intercalate DNA (fig. S5B). Previously, a hydrazide-based small molecule (O8) was reported to inhibit OGG1 with similar potency as TH5487 (19). O8 was found to inhibit catalytic imine formation in OGG1 (19), and we observed an increase in the potency of O8 by omitting APE1 from the reaction, in contrast to TH5487 (table S4). APE1 readily released fluorescence from a natural AP site but only partially from an AP-site substrate preincubated with O8 (fig. S5C). Thus, TH5487 primarily inhibited the DNA glycosylase activity of OGG1, whereas O8 appeared to interfere with downstream β -lyase activity. To further validate OGG1 inhibition by TH5487, we performed electrophoretic mobility shift assays, where OGG1 bound to 8-oxoG:C-containing duplex oligonucleotide in a concentration-dependent manner (fig. S4C). The amount of OGG1-DNA complexes decreased in a dose-dependent manner upon addition of TH5487 (Fig. 1E), demonstrating that TH5487 precludes OGG1 from binding oxidized DNA in vitro.

TH5487, but not the inactive analogs TH2840 and TH5411, increased the melting temperature for OGG1 in a concentration-dependent manner (Fig. 1F). Thus, TH5487-mediated protein destabilization did not account for the observed decrease in enzyme activity, suggesting that TH5487 binds OGG1 similarly to 8-oxoG extruded from DNA. Supporting this, treatment with TH5487 resulted in a lower deuteration for all peptides forming the active site cavity (Fig. 1G and table S5). Thus, TH5487 is a potent and selective active site inhibitor that prevents OGG1 from binding to its DNA substrate.

To identify the precise binding site for this class of inhibitors, we determined the x-ray crystal structure of mouse OGG1 in complex with the more soluble analog TH5675 (Fig. 1H; figs. S4 and S6, A to C; and table S6). TH5675 bound the active site (fig. S6D), albeit differently from the natural substrate (fig. S6E). Notably, the iodo-phenyl tail of TH5675 occupied the deeper hydrophobic pocket flanked by Phe³¹⁹, Cys²⁵³, and Met²⁵⁷ and took the place of the 8-oxoguanine base. The central piperidyl linker was stabilized by hydrogen bonds with the catalytic Lys²⁴⁹ and the backbone of Gly⁴², the residue that distinguishes 8-oxoguanine from guanine. The benzimidazolone core interacted with a lipophilic exosite, stabilized by Ile¹⁵² and Leu³²³ in addition to a π -stacking interaction with His²⁷⁰ (20). Notably, the Asp³²² side chain was within hydrogen-bond distance of the solvent accessible amine, which corresponds to the bromine atom in TH5487 (fig. S6F). These interactions

were the result of a local conformational change in which the active site closed around the ligand (fig. S6G and movie S1).

For OGG1 inhibitors to be pharmacologically useful, they need to engage and inhibit OGG1 in cells. TH5487 increased the melting temperature of OGG1 in human cells (Fig. 2A), demonstrating that TH5487 engaged its intended target in living cells and protected it from thermal denaturation. Furthermore, TH5487 impaired repair of genomic 8-oxoG induced by KBrO₃. TH5487 caused a significant increase in genomic 8-oxoG after 2.5 hours (Fig. 2, B and C), and at 24 hours, 50 ± 8% of the 8-oxoG remained in the TH5487-treated cells (Fig. 2C), without disrupting proliferation (fig. S7A). Thus, genomic 8-oxoG and TH5487 were well tolerated by cells. Furthermore, the decrease in genomic 8-oxoG was a result of repair processes and not cellular replication. To further validate target engagement, we assessed the chromatin dynamics of OGG1-GFP (green fluorescent protein) fusion proteins. Cells were treated with KBrO₃ and released into medium containing TH5487 or dimethyl sulfoxide (DMSO). Consistent with previous reports (21), OGG1-GFP fusion proteins were immobilized at genomic DNA lesions introduced by KBrO₃. Treatment with TH5487 increased the nuclear mobility of OGG1-GFP both 3 and 5 hours after KBrO₃ exposure (Fig. 2, D and E, and fig. S7, B and C), suggesting that TH5487 prevented OGG1 binding to its genomic substrate in living cells.

OGG1 binds 8-oxoG at gene regulatory regions to mediate transcriptional activation in response to inflammatory stimuli (3, 7–11). In the absence of functional OGG1, a decreased inflammatory response is observed (3,12–16,22). Because TH5487 prevents OGG1 from binding 8-oxoG in DNA, we examined if TH5487 could suppress proinflammatory gene expression. In line with previous observations (12), human embryonic kidney (HEK) 293T cells lacking OGG1 displayed a reduced induction of *CXCL1* [chemokine (C-X-C motif) ligand 1] mRNA after tumor necrosis factor- α (TNF α) stimulation (Fig. 2F and fig. S7, D and E). Treatment with 5 μ M TH5487 decreased *CXCL1* expression by >50% in wild-type but not in *OGG1*-knockout cells (Fig. 2F). Thus, the compound may be used to specifically inhibit OGG1-dependent proinflammatory gene expression. Because respiratory epithelium is a key orchestrator of pulmonary innate immune responses (23), we stimulated a murine airway epithelial cell line (MLE12) with TNF α (24), which increased the expression of an array of proinflammatory cytokines as well as C-C and C-X-C chemokines (Fig. 3, A to C, and fig. S8). Importantly, TH5487 decreased the expression of the same genes to near pretreatment levels (Fig. 3, C and E, and figs. S8 to S12). Inhibition was dose-dependent (Fig. 3D and fig. S10) and also observed with the potent inflammatory agent lipopolysaccharide (LPS) (25) (Fig. 3F and figs. S11 and S12). Crucially, TH5487 decreased TNF α - and LPS-induced gene expression in diploid human small-airway epithelial cells (hSAECs) as well (Fig. 3, G to I, and figs. S9, S10, and S12).

ROS generate a localized increase in OGG1 substrates in guanine-rich promoter regions (4, 6, 9,10), including proinflammatory genes (3,4,12). Emerging evidence suggests that OGG1 binding to gene regulatory regions exerts an epigenetic role for 8-oxoG, causing OGG1 to act as a modulator of gene expression (3,4,6–11). Guanine oxidation leads to sequential recruitment of OGG1 and downstream transcriptional effectors (3,8–11), such as nuclear factor κ B (NF- κ B), which is the main driver of both TNF α - and LPS-induced

proinflammatory gene expression (26). Consistent with the observation that TH5487 prevents OGG1 from engaging damaged DNA in vitro and in cells (Figs. 1E and 2, D and E), we observed that TH5487 decreased the recruitment of OGG1 to regulatory regions of proinflammatory cytokines in TNF α -challenged cells (Fig. 3J). Consequently, binding of NF- κ B to the same regulatory regions was significantly decreased by TH5487 in the chromatin of TNF α -exposed cells (Fig. 3K) and to its recognition sequence in nuclear extracts from mouse and human cells by TH5487 (Fig. 3L and fig. S13, A to C). In the presence of OGG1, TH5487 decreased NF- κ B occupancy on 8-oxoG-containing DNA, whereas TH5487 alone was unable to inhibit NF- κ B (fig. S13, D and E). Thus, TH5487 decreases proinflammatory gene expression by perturbing DNA occupancy of NF- κ B and potentially other OGG1-dependent transacting factors (3, 8–11). TH5487 had no effect on the release of NF- κ B from its inhibitory complex (fig. S14, A to C) but inhibited inflammatory gene expression similar to BMS-345541, an I κ B kinase inhibitor (27) (fig. S15A). Thus, both TH5487 and BMS-345541 inhibit NF- κ B function: TH5487 by preventing NF- κ B binding to promoters (Fig. 3K) and BMS-345541 by inhibiting NF- κ B activation (fig. S14, A to C). This results in the same readout in the form of diminished induction of proinflammatory genes. The previously developed OGG1 inhibitor O8 (19) did not affect gene expression (fig. S15A), possibly because, in contrast to TH5487, it allows OGG1 binding to damaged DNA (19) (fig. S15, B and C).

In addition, TH5487 is metabolically relatively stable and well tolerated in mice (fig. S16A and tables S7 to S10). To assess whether TH5487 could down-regulate chemotactic (C-C and C-X-C) mediators (28) in vivo, we challenged mouse lungs with TNF α and profiled the gene expression of proinflammatory mediators. TNF α robustly induced the expression of pulmonary proinflammatory genes, but a prophylactic injection of TH5487 decreased the expression levels (Fig. 4, A and B). Challenge with TNF α or LPS induced the robust recruitment of neutrophils to the airways, which was decreased by up to $85 \pm 5\%$ by the prophylactic intraperitoneal administration of TH5487 (Fig. 4C and fig. S16, B to G). We then administered TH5487 at different time points before or after challenge with TNF α and found that TH5487 reduced the pulmonary neutrophil count even when administered up to 9 hours after TNF α challenge (Fig. 4D and fig. S17). Thus, TH5487 is efficacious in vivo, suggesting that the compound could be used for the treatment of inflammatory conditions. Finally, another potent and structurally distinct OGG1 inhibitor was recently published (29). When tested, this compound had comparable anti-inflammatory effects (fig. S18).

Thus, we have developed a pharmacologically useful OGG1 inhibitor that is a potent and selective active site binder that prevents OGG1 from engaging damaged DNA in vitro and in cells, resulting in decreased proinflammatory gene expression by a mechanism that is distinct from other established therapeutic agents (fig. S19). This is translated into a reduced neutrophil infiltration in mouse lungs challenged with TNF α or LPS, demonstrating that OGG1 inhibition may be a potentially useful strategy for the treatment of inflammation.

Supplementary Material

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Fig. 1. Development and validation of OGG1 inhibitors.

(A) A fluorophore and a quencher on opposite strands are separated upon OGG1-mediated excision of 8-oxoA and APE1 incision at the resulting apurinic site, causing a local melting of the DNA helix. (B) Excision of 8-oxoA:C, but not undamaged substrates, by OGG1 in the presence of APE1. Data are presented as averages $\pm$ SD of three technical replicates from four independent experiments. A.U., arbitrary units. (C) Chemical structures of the OGG1 inhibitors described herein. (D) Inhibition curves. 0.8 nM OGG1 and 2 nM APE1 were incubated with 10 nM OGG1 substrate and different concentrations of the indicated

compounds. Data are presented as averages of four technical replicates from at least two independent experiments ($n = 2$ for TH2840 and TH5411, $n = 33$ for TH5487). (E) TH5487 precludes binding of OGG1 to damaged DNA. Ten nM of an OGG1-substrate duplex oligonucleotide was incubated with 100 nM OGG1 and the indicated concentrations of TH5487. This prevented the formation of OGG1-DNA complexes in a dose-dependent manner. The figure is representative of three independent experiments. (F) Differential scanning fluorimetry. OGG1 was incubated with SYPRO Orange and a dilution series of OGG1 inhibitors. TH5487, but not inactive analogs TH2840 and TH5411, confers the thermal stabilization of OGG1. Data are presented as averages $\pm$ SD of three technical replicates from three independent experiments. T_m , melting temperature. (G) Differences in deuterium uptake superimposed on an OGG1 model upon TH5487 binding (Protein Data Bank 1EBM). Colored regions show peptides protected from deuterium exchange. The molecular surface of TH5487 is shown as a semitransparent surface, and DNA is displayed as a ribbon. (H) X-ray crystal structure of mouse OGG1 (gray) in complex with ligand (yellow). N and C termini are labeled. On the right is a close-up view of ligand binding. Important amino acid residues are marked; hydrogen-bond interactions are shown with black dashed lines. The view in (H) differs from the one in (G). Single-letter abbreviations for the amino acid residues are as follows: C, Cys; D, Asp; F, Phe; G, Gly; H, His; I, Ile; K, Lys; M, Met; P, Pro; and Q, Gln.

Fig. 2. TH5487 engages OGG1 in cells, inhibits DNA repair, and alters OGG1 chromatin dynamics.

(A) Cellular thermal shift assay. Jurkat A3 cells were treated with 10 μ M TH5487, and OGG1 thermal stability was analyzed by immunoblotting. Addition of 10 μ M TH5487 to cultured cells increased the melting point of OGG1 by 3 $^{\circ}$ C ($n = 2$ independent experiments). Actin was used as a loading control. (B) Induction of genomic 8-oxoG by KBrO₃. Duplicate cultures of Jurkat A3 cells were treated for 1 hour with 20 mM KBrO₃, and the amount of 8-oxoG in genomic DNA was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). KBrO₃ induced a >10-fold increase in genomic 8-oxoG. Data are presented as averages $\pm$ SD of four replicates from two independent experiments. dG, deoxyguanosine. (C) Repair kinetics of genomic 8-oxoG. Cells treated with 20 mM KBrO₃ for 1 hour were washed and released into medium containing 10 μ M TH5487 or 0.1% DMSO. Duplicate samples were taken at the indicated time points, and the genomic content of 8-oxoG was determined as in (B). TH5487 induced a notable delay in repair kinetics at 2.5-, 5- and 24-hour time points. Data are presented as averages $\pm$ SD of four replicates from two independent experiments. (D) Fluorescence recovery after photobleaching (FRAP). Jurkat A3 cells expressing OGG1-GFP were treated with 16 mM KBrO₃, washed, and released into medium with 10 μ M TH5487 or 0.1% DMSO. A nuclear region was bleached, and recovery of fluorescence after photobleaching was recorded. Representative false-color images of DMSO- and TH5487-treated cells are shown. Dashed outlines indicate bleached areas. Scale bar, 5 μ m. (E) Quantification of FRAP experiments. Ten μ M TH5487 increased the nuclear mobility of OGG1-GFP at 3 and 5 hours after KBrO₃ treatment. Quantifications

of two (0-hour) or three (3- and 5-hour) independent experiments are shown. RFU, relative fluorescence units. (F) TH5487 inhibits TNF α -induced *CXCL1* gene expression in wild-type, but not in *OGG1*-knockout (KO), HEK293T cells. Cells were treated with 0.05% DMSO or 5 μ M TH5487 for 1 hour and TNF α (20 ng/ml) for 30 min. *CXCL1* mRNA levels were determined with quantitative polymerase chain reaction (qPCR). Data are presented as averages $\pm$ SD from three independent experiments. For (B), (C), and (F), ** P < 0.01, *** P < 0.001, **** P < 0.0001, and NS is not significant, using unpaired two-sided Student's t test.

Fig. 3. Inhibition of proinflammatory gene expression and inflammation by TH5487, an active site binder of OGG1.

(A to C) The effect of TH5487 on basal (A) and TNF α -induced expression of an array of proinflammatory cytokines, chemokines, and receptors [(B) and (C)] in mouse airway epithelial cells (MLE12). Data analyses were performed according to the manufacturer's instructions using their web-based software package (www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/). **(D)** The dose-dependent inhibition of TNF α -induced *Tnf* mRNA levels by TH5487 in MLE 12. **(E and F)** TH5487 inhibits the TNF α - (E) and LPS- induced (F) expression of proinflammatory genes in MLE 12. **(G)** Dose- dependent inhibition by TH5487 of TNF α -induced expression of *TNF* in hSAECs. **(H and I)** TH5487 inhibits TNF α - (H) or LPS-induced (I) expression of proinflammatory genes in hSAECs. In (A), (B), (C), (E), (F), (H), and (I), parallel cultures of cells were treated with solvent or TH5487 (5 μ M) for 1 hour and TNF α (20 ng/ml for 30 min) or LPS (100 ng/ml

for 1 hour) was added. In (D) and (G), decreasing concentrations of TH5487 were added before TNF α (20 ng/ml for 30 min). Changes in mRNA levels were determined by quantitative real-time PCR. Data are presented as averages $\pm$ SD from at least three independent experiments. (J) TH5487 decreases binding of OGG1 to promoters in chromatin. (K) TH5487 perturbs DNA occupancy of NF- κ B in chromatin. In (J) and (K), data are presented as averages $\pm$ SD from four independent experiments, and MLE 12 cells were treated with solvent or 5 μ M TH5487 for 1 hour and exposed to TNF α (20 ng/ml) for 30 min. Chromatin was immunoprecipitated using antibody to epitope-tagged OGG1, or the p65 subunit of NF- κ B. Fold changes in OGG1 and NF- κ B binding to the indicated proximal promoter regions were determined by qPCR. (L) TH5487 perturbs binding of NF- κ B to 8-oxoG-containing synthetic DNA in nuclear extracts from MLE 12 or hSAEC cells. p50-p65, heterodimer of NF- κ B; p50-p50, homodimer of NF- κ B. Images are representative of three independent experiments. In (D) to (I), * P <0.05, ** P <0.01, *** P <0.001, and NS is not significant, using unpaired two-sided Student's t test.

Fig. 4. TH5487 suppresses proinflammatory gene expression and lung inflammation in mice. (A and B) Groups of mice were treated intraperitoneally with TH5487 (30 mg/kg) or vehicle, and lungs were TNF α -challenged intranasally (20 ng/ml). The bars represent expression levels of mRNAs pooled from lungs of six individual mice. Target gene signals were normalized to housekeeping genes, and all data analyses were performed according to the manufacturer's instructions using their web-based software package (www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/) ($n = 1$ experiment). (C) Dose dependent inhibition of TNF α -induced neutrophil infiltration by TH5487. Mice (50% female and 50% male) were treated intraperitoneally with increasing doses of TH5487 and challenged intranasally with TNF α . Sixteen hours after challenge, mice were euthanized and lavaged. Neutrophil numbers in bronchoalveolar lavage fluid were determined in a blinded fashion. (D) TH5487 interrupts TNF α -induced ongoing inflammatory processes. Randomly selected groups of mice (50% female and 50% male) were challenged intranasally with vehicle or 20 ng TNF α per lung, with TH5487 administered intraperitoneally 1 hour before or 3, 6, or 9 hours thereafter. Sixteen hours after TNF α stimulation, mice were euthanized and lavaged. The levels of neutrophil infiltration were assessed as described for (C). In (C) and (D), *** $P < 0.001$, using unpaired two-sided Student's t test.