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Potent and Selective Inhibitors of 8-Oxoguanine DNA Glycosylase

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Abstract

The activity of DNA repair enzyme 8-oxoguanine DNA glycosylase (OGG1), which excises oxidized base 8-oxoguanine (8-OG) from DNA, is closely linked to mutagenesis, genotoxicity, cancer, and inflammation. To test the roles of OGG1-mediated repair in these pathways, we have undertaken the development of noncovalent small-molecule inhibitors of the enzyme. Screening of a PubChem-annotated library using a recently developed fluorogenic 8-OG excision assay resulted in multiple validated hit structures, including selected lead hit tetrahydroquinoline **1** (IC₅₀ = 1.7 μ M). Optimization of the tetrahydroquinoline scaffold over five regions of the structure ultimately yielded amidobiphenyl compound **41** (SU0268; IC₅₀ = 0.059 μ M). SU0268 was confirmed by surface plasmon resonance studies to bind the enzyme both in the absence and in the presence of DNA. The compound SU0268 was shown to be selective for inhibiting OGG1 over multiple repair enzymes, including other base excision repair enzymes, and displayed no toxicity in two human cell lines at 10 μ M. Finally, experiments confirm the ability of SU0268 to inhibit OGG1 in HeLa cells, resulting in an increase in accumulation of 8-OG in DNA. The results suggest the compound SU0268 as a potentially useful tool in studies of the role of OGG1 in multiple disease-related pathways.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b09316.

Experimental details and characterization data (NMR, MS) for all synthesized compounds (PDF)

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Notes

The authors declare the following competing financial interest(s): Y.T. and E.T.K. are inventors on a patent application related to this work.

INTRODUCTION

The consequences of DNA damage are important to many biological pathways and are central to mutagenesis, genotoxicity, and tumorigenesis.^{1–6} Among the most frequent causes of damage in DNA is 8-oxoguanine (8-OG), which is generated by reactive oxygen species arising from metabolism, or exposure to agents that induce oxidative stress.^{5–17} Because 8-OG is mispaired frequently with adenine during DNA replication, it is highly mutagenic,¹⁸ and the frequency of guanine oxidation combined with this mispairing causes it to be the greatest single source of mutations in the cell.¹⁵ Mutations that occur in proto-oncogenes can initiate tumorigenesis,¹⁹ and the presence of 8-OG in DNA can also be harmful to cell growth by leading to DNA double-strand breaks.²⁰

The primary enzyme for repairing 8-OG in DNA is 8-oxoguanine DNA glycosylase (OGG1), which functions via a base excision repair (BER) mechanism.²¹ The enzyme exists in both the nucleus and mitochondria, repairing DNA damage in both subcellular locations.²² It recognizes 8-OG in double-stranded DNA and cleaves the glycosidic bond, releasing the 8-OG free base and generating an abasic deoxyribose in the DNA.²¹ This abasic site is then further processed by lyase activities of the enzyme itself or the AP lyase enzyme, ultimately leading to strand cleavage.^{20,23,24} Additional enzymes in the BER pathway can then complete the damage repair, regenerating intact DNA with correctly paired bases.²¹

Because of the importance of OGG1 to DNA mutations and genotoxicity, a good deal of study has been devoted to investigating how varied levels of this activity can affect disease pathologies. OGG1-deficient mice display elevated levels of genomic 8-OG and increased mutations, highlighting the importance of this enzyme in maintaining genome integrity.^{25,26} OGG1 mutants with abolished or decreased 8-OG repair activity have been found in many tumor specimens.^{27,28} A common OGG1 polymorphism, S326C, which lowers BER activity, is positively associated with frequency of cancers of the digestive system and the lung.^{29–31} In addition to its prominent association with genetic mutations leading to malignancies, OGG1-mediated repair of 8-OG has been associated with inflammation-related pathologies as well.³² 8-OG is known to act as a signaling molecule that modulates activity of several GTPases.³³ Downregulated OGG1 activity results in decreased lung inflammation in murine allergy models.³² Furthermore, polymorphisms in the *OGG1* gene have been associated with rheumatoid arthritis.³⁴

Taken together, the data suggest strong links between OGG1 and multiple disease states. For further studies of these relationships, it would be desirable to have small molecule inhibitors of the enzyme, which could be useful as tools to study OGG1-related pathways and pathologies in cellular and animal models. The only previously known small molecule inhibitors of OGG1 were reported recently by Lloyd, who described simple hydrazine and hydrazone derivatives that inhibited the enzyme as measured by an assay that measures DNA strand cleavage subsequent to base excision.³⁵ Since hydrazones can spontaneously hydrolyze,³⁶ and hydrazines are known to react generally with abasic sites in DNA,^{37,38} such classes of compounds may raise questions of stability and specificity. In general, the use of BER assays that measure DNA cleavage rather than the excision event may result in identification of hit compounds that do not act by inhibiting initial base excision.

For these reasons, discovery and development of highly potent, selective, and stable small-molecule inhibitors of OGG1 remain an important goal, and this goal would be aided by an assay that directly measures excision. In an effort to directly measure the base excision activity of OGG1, we recently developed fluorogenic probe (OGR1) that can detect the removal of 8-OG in real time (Figure 1).³⁹ In this probe, 8-OG acts as a fluorescence quencher of a neighboring fluorescent DNA base, and enzymatic excision of the 8-OG renders the probe emissive. The fluorescent signal then yields a quantitative measurement of OGG1 activity.

Herein, we describe the development of small-molecule OGG1 inhibitors using this fluorogenic assay in high throughput, leading to the identification of a tetrahydroquinoline scaffold with significant inhibitory activity. We report structure–activity relationships of a broad set of derivatives as potential inhibitors of the enzyme, and we describe the properties of the potent and selective inhibitor SU0268 that resulted from this work.

MATERIALS AND METHODS

General Information

¹H NMR spectra were recorded on Varian Inova 300 (300 MHz) spectrometers. The chemical shifts were reported in parts per million (δ) relative to internal standard TMS (0 ppm) for CDCl₃. The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; dt, m, multiplet; q, quartet. The coupling constants, *J*, are reported in hertz (Hz). ¹³C NMR spectra were obtained by using a Varian Inova 300 instrument. CDCl₃ was used as an NMR solvent (Acros Organics). Low-resolution electrospray ionization mass spectra (ESI-MS) were measured on an ACQUITY UPLC system (Waters). High-resolution mass spectra (HRMS) were measured via ESI on a micrOTOF-Q II instrument (Bruker). Ultraviolet spectra were measured on a Varian Cary 300 spectrophotometer. Fluorescence intensities were measured on Fluoroskan Ascent microplate fluorometer (Thermo Scientific). The OGR1 probe was synthesized by using an Applied Biosystems 394 DNA/RNA synthesizer. cLogP and tPSA were calculated by ChemDraw. Analytical thin-layer chromatography was performed on ready-to-use plates with silica gel 60 (Merck, F254). Flash column chromatography was performed over Fisher Scientific silica gel (grade 60, 230–400 mesh). All reagents were weighed and handled in air and backfilled under argon at room temperature. Unless otherwise noted, all reactions were performed under an argon

atmosphere. All chemicals were purchased from Acros Organics, Alfa Aesar, Ark Pharm, Asta Tech, Combi-Blocks, Enamine, Matrix Scientific, Sigma-Aldrich, and TCI and used without further purification.

Synthesis of Compounds 1–44

Details of the synthesis and characterization of these analogues and their synthetic intermediates are given in the Supporting Information file.

General Procedure for OGG1 Inhibitor Assay

BSA, hOGG1, and NEBuffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9 at 25 °C) were purchased from New England Biolabs and used as received. Ultrapure distilled water (Invitrogen) was purchased and used for the assay.

Synthesized compounds and hOGG1 (100 nM) were incubated in NEBuffer 4 (1 X) with BSA (1 X) at 37 °C (15 min) in 100 μ L reaction volumes in a black 96-well plate. After that, OGR1 probe³⁹ (1.2 μ M) was added to the reaction mixture. Fluorescence at 460 nm was measured on a Thermo Fluoroskan Ascent FL fluorescence plate reader (λ_{ex} = 355 nm). The slope of initial rate (12 min) was calculated, and percent of control was used for inhibition activity.

For the HTS, the assay was optimized for 384-well plates (low volume, black solid plates, Greiner cat. no. 784076). For the HTS assay, 5 μ L of 100 nM hOGG1 was dispensed using a FlexDrop dispenser (PerkinElmer) in NEBuffer 4. Next, library plates containing compounds at 2 mM in DMSO were used to transfer 100 nL of the DMSO solution to the assay plate using an acoustic dispenser (EDC Biosystems, ATS). After a 15 min incubation at 37 °C, the enzyme reaction was started by adding 5 μ L of 1.6 μ M OGR1 in NEBuffer 4 using the FlexDrop. Final concentrations of OGG1 and OGR1 were then 50 nM and 0.8 μ M, respectively. The reaction was incubated at 37 °C for 30 min, and the fluorescence was determined on a PerkinElmer Envision microtiter plate reader (λ_{ex} = 340 nm and λ_{em} = 450 nm using Lance/Delfia dichroic mirror of 412 nm). See Supporting Information for further details.

Screening of Enzyme Selectivity

Details of assays for MTH1, dUTPase, NUDT16, AlkBH2, AlkBH3, and SMUG1 are given in the Supporting Information.

Surface Plasmon Resonance (SPR) Studies

Recombinant His-tagged OGG1 (Creative Biomart) was exchanged to phosphate buffered saline (PBS). Limited biotinylation of OGG1 surface lysines was performed at 4 °C for 20 min using EZ-Link Sulfo-NHS-LC-Biotin (Thermo Scientific) and exchanged back to PBS on a desalting column to quench the reaction. All SPR studies were performed on a Biacore 8K instrument (GE Healthcare Life Sciences) using 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT, 2% dimethyl sulfoxide (DMSO), and 0.005% Tween, pH 7.9 as the running buffer. A total of 3000 resonance units (RU) of

biotinylated OGG1 was captured on streptavidin sensor chip SA (GE Healthcare Life Sciences). Following capture, any remaining streptavidin sites on both the reference and active channels were blocked with biocytin. A 1:3 dilution series of compound **41** (highest concentration 4 μM), prepared to match the running buffer, was flowed over OGG1. All sensorgrams shown are reference subtracted with solvent correction procedures implemented. Data were fit to both steady state and a 1:1 kinetic model using Biacore evaluation software.

Michaelis–Menten Kinetics Studies

For Michaelis–Menten curves, inhibitor **41** and hOGG1 (100 nM) were incubated in NEBuffer 4 (1X) with BSA (1X) at 37 °C (15 min) in 100 μL reaction volumes in a black 96-well plate. After that, OGR1 (0.8–10 μM) was added to the reaction mixture. Fluorescence at 460 nm was measured on a Thermo Fluoroskan Ascent FL fluorescence plate reader (λ_{ex} = 355 nm). The initial rate (10 min) was calculated.

Lineweaver–Burk plots were derived from the Michaelis–Menten curves. K_{m} and V_{max} were calculated from the equation. k_{cat} was calculated from $k_{\text{cat}} = V_{\text{max}}/[\text{enzyme}]$.

DNA Binding Studies

Abasic DNA:NEBuffer 4 (1 X), 1 μM abasic DNA, 2 μM **41** (1% DMSO) were included in 1600 μL reaction volumes with 1 cm path-length quartz cells. The UV absorbance at 260 nm was measured on a Varian Cary 300 as temperature was raised 1 °C/min. The T_{m} value was calculated by Meltwin.

For the OGR1 probe, PBS buffer (0.1 X), 1 μM OGR1 probe, 2 μM **41** (10% DMSO) were included in 800 μL reaction volumes with 1 cm path-length quartz cells. The UV absorbance at 260 nm was measured on a Varian Cary 300 as temperature was raised 1 °C/min. The T_{m} value was calculated by Meltwin.

Toxicity via MTT Assay

HEK293T cells (1.6×10^4 cells per well) and HeLa cells (concentration of 1.2×10^4 cells per well) were seeded to 96-well plate in supplemented DMEM culture medium (10% FBS, 100U Penicillin/Streptomycin) and incubated for 30 h (HEK293T cells) and 16 h (HeLa cells) at +37 °C, 95% humidity and 5% CO_2 . They were incubated in 100 μL of the fresh medium at concentrations of 100 μM , 10 μM , 1 μM , 100 nM, and 10 nM of compound **41** for 24 h at +37 °C, 95% humidity, and 5% CO_2 , in supplemented DMEM culture medium. After the incubation period, 10 μL of the MTT labeling reagent (final concentration 0.5 mg/mL) was added to each well, and incubated at +37 °C, 95% humidity, and 5% CO_2 . 100 μL of the solubilization solution was added into each well, followed by incubation for 20 h at +37 °C, 95% humidity, and 5% CO_2 . Absorbance of the samples was measured by using a microplate reader (Tecan Infinite M1000) at 550 and 650 nm. Compound **41** was provided in concentration of 2 mM in DMSO that determined final concentration of DMSO in 100 μM at the level of 5%. In the remaining samples (10 μM , 1 μM , 100 nM, 10 nM) concentration of DMSO was at the level of 1%. The controls were prepared as follows: Ctrl, cells cultured only in the supplemented DMEM; Ctrl 5% DMSO, cells cultured in 5% DMSO in the

supplemented DMEM; Ctrl 1% DMSO, cells cultured in 1% DMSO in the supplemented DMEM.

Detection of 8-OG in Cell Line by LC-MS/MS

HeLa cells (2×10^6 cells per well) were seeded in 10 cm dishes in supplemented DMEM culture medium (10% FBS, 100U penicillin/streptomycin) and incubated for 12 h (HeLa cells) at +37 °C, 95% humidity, and 5% CO₂. They were incubated in 10 mL of the fresh medium at a concentration of 5 μ M and 0.5 μ M of compound **41** for 24 h at +37 °C, 95% humidity, and 5% CO₂, in supplemented DMEM. Every 6 h fresh medium with compound **41** was added. Control HeLa cells were cultured in supplemented DMEM culture medium. For positive oxidative damage controls, we cultured HeLa cells in the supplemented DMEM medium with 0.5 mM H₂O₂ and 0.3 mM CrCl₃. After incubation, all cells were washed twice with PBS and harvested for DNA isolation. DNA isolation was performed with Quick-DNA Miniprep Plus Kit (Zymo Research) according to the manufacturer's protocol. The concentration of DNA eluted in nuclease-free water was measured on a NanoDrop One instrument (Thermo Scientific). The DNA (5 μ g) was dried and then digested with 0.02 U spleen II nuclease (Worthington) and 0.4 U micrococcal nuclease (Sigma-Aldrich) in 20 mM succinate buffer containing 10 mM CaCl₂ for 16 h at 37 °C. The hydrolysate was treated with 0.4 μ g of RNase P1 (Sigma-Aldrich) in 78 mM ammonium acetate buffer (pH 4.5) and 10 mM bicine-NaOH buffer (pH 9.7) containing 10 mM MgCl₂, 10 mM dithiothreitol, and 1 mM spermidine for 6 h at 37 °C. The final volume of the mixture was 25 μ L. Next, 75.5 μ L of internal standard was added to all samples and blank sample to a total volume of 100 μ L. One of the blank samples was prepared without internal standard. The reaction mixtures were filtered through Amicon Ultra 0.5 mL 3 kDa cutoff filters (Millipore). The set of 12 spiking solutions, containing dG, dC, 8oxodG, was prepared according to the digestion procedure for DNA. The detection range covered by spiking solution was: 0.0625 ng/mL to 100 ng/mL for 8-oxodG, and 2.5 ng/mL to 5.97 μ g/mL for dG. Additionally, in the LC-MS/MS analysis internal standards of [¹⁵N₅] dG and [¹⁵N₃] dC were used. The nucleosides were separated on a Synergi 4 μ m Fusion column, RP 80 Å, 150 $\times$ 2 mm, at 30 °C with 10 μ L injection volume, and flow rate of 200 μ L/min. Separation was performed using following program: 0–3 min 100% phase A (0.1% formic acid in water), 9 min 60% phase A and 40% phase B (0.1% formic acid in acetonitrile), 12 min 10% phase A, 12.1–15 min 100% phase A. Collected values of 8-oxodG content (ng/mL) were normalized to the amount of dG for all replicates.

Inhibition of OGG1 Activity in Cell Lysates by SU0268

HeLa and MCF-7 cells were seeded in 15 cm dishes in supplemented DMEM culture medium (10% FBS, 100U Penicillin/Streptomycin) (Gibco) and incubated for 48 h at +37 °C, 95% humidity, and 5% CO₂ to full confluence. Cells were scraped, washed with 1X PBS (pH 7.4), and decanted. For each cell culture the packed cell volume (PCV) was estimated. The 5X PCV of lysis buffer (10 mM HEPES pH 7.9 (Gibco), with 1.5 mM MgCl₂ (Sigma-Aldrich), 10 mM KCl (Sigma-Aldrich), 1 mM DTT (Invitrogen), and 1X protease inhibitor cocktail (Roche)) was used to resuspend cells. The suspension was incubated on ice for 15 min to allow cells to swell and then centrifuged for 5 min at 420g at 4 °C. The cell pellet was resuspended in 2X PCV of lysis buffer and lysed using a syringe with a narrow-

gauge (No. 25) hypodermic needle. Disrupted cells were centrifuged 20 min at 11000*g*, at 4 °C. The collected supernatant was the cytoplasmic fraction. The nuclei pellet was resuspended in 2/3X PCV of extraction buffer (20 mM HEPES pH 7.9 (Gibco), with 1.5 mM MgCl₂ (Sigma-Aldrich), 0.42 M NaCl (Sigma-Aldrich), 0.2 mM EDTA (Invitrogen), 25% (v/v) Glycerol (Sigma-Aldrich), 1 mM DTT (Invitrogen), and 1X protease inhibitor cocktail (Roche)) and shaken for 60 min at 4 °C. The suspension was centrifuged for 5 min at 21000*g* at 4 °C, and the supernatant containing nucleoplasmic fraction was collected. Two volumes of cytoplasmic fraction were mixed with one volume of nucleoplasmic fraction, and this protein mixture was used in the experiment. Concentration of proteins was measured using the Bradford Protein Assay (Biorad) according to the manufacturer's protocol. Reactions were performed with a total volume of 80 μ L, containing 100 ng/ μ L total cell protein, 2 μ M pOGR1 probe, and 10 mM MgCl₂, and were incubated for 24 h at 37 °C in a black 384-well plate. Fluorescence was measured at 460 nm using a Fluoroscan Ascent FL plate reader (Thermo Scientific) and excitation wavelength 355 nm.

Procedure for CACO-2 Assay

Measurements were carried out by Absorption Systems LLC. See Supporting Information for experimental details.

RESULTS AND DISCUSSION

Small-Molecule Screening for OGG1 Inhibition

The identification of OGG1 inhibitors was initiated with a high-throughput screen of the PubChem-annotated library compounds using hOGG1 enzyme and our originally developed OGR1 probe assay, using recombinant human OGG1. Approximately 26 000 compounds were chosen for initial screening, and the assay was carried out at a single 20 μ M concentration (Figure 2). The high-throughput screen showed good performance with $Z' = 0.68 \pm 0.1$ and led to over 350 initial hit compounds using a threshold of >35% inhibition, which was >3 s.d. of the maximum enzyme activity controls treated with DMSO vehicle alone. The hit compounds showed varied chemical structures and were then further validated by titration over an 8-fold concentration range to determine IC₅₀ values. The assay yielded several validated hit compounds, from which we selected compound **1**, a tetrahydroquinoline sulfonamide derivative, for further study. Compound **1** did not score as active in another fluorescence enzyme assay which used the same fluorescence detection protocol,⁴⁰ thus confirming that its activity was not a false positive resulting from inherent fluorescence. In addition, compound **1** (annotated in PubChem as CID:7517554) has been tested in 84 assays. Of these assays, only two listed the compound as active, with no IC₅₀'s below 10 μ M, supporting a selective compound profile.

Our general strategy for structural optimization of scaffold **1** is shown in Figure 2. The chemical structure of **1** was divided into five fragments, A–E: Fragment B consists of the core tetrahydroquinoline structure, and A is a substituent on the nitrogen atom of this core structure. Fragment C, a sulfonamide, is a linker between core structure B and fragment D, a benzene ring. Fragment E is a substituent of this aromatic ring. We varied these five

fragments to investigate their influences on OGG1 base excision as measured by the OGR1 assay.

Structural Optimization

For the initial optimization studies, we synthesized analogues of hit compound **1** with varied substituents (R^1) on the nitrogen atom of the tetrahydroquinoline skeleton (Table 1). Assays of these compounds were carried out over a three-log concentration range to differentiate weak inhibitors from stronger ones. Lead compound **1** (with acetamide substituent as R^1) showed significant inhibition of OGG1 at 20 μ M and ca. 50% inhibition at 2 μ M, but yielded little inhibition activity at 200 nM. Homologation of acetamide to ethylamide (**2**) had little effect on inhibition, while larger substituents (**3–6**) lowered the activity. Interestingly, however, cyclopropylamide analogue **7** showed significantly more potent inhibition activity than the lead compound (see Table 6, below) and was also more active than the closely related isopropyl variant **5**. A slightly larger ring (cyclobutyl compound **8**) showed somewhat lower potency. We proceeded to other R^1 substituents, varying the carbonyl itself. Sulfonamide analogue **9**, urea analogue **10**, and reduced analogue **11** were prepared and assayed. However, these compounds showed little or no activity at 20 μ M. These results establish that the carbonyl group on the tetrahydroquinoline nitrogen atom is highly important for activity, and among them, the cyclopropylamide group was the optimal substituent for R^1 .

Next, we examined fragment B, the core structure of the initial hit compound (Table 2). Acyclic compound **12** and 5-membered-ring dihydroindole **13** showed low activity. The 7-membered-ring analogue **14** was also examined, but displayed lower inhibition activity than the 6-membered-ring analogue **7** shown in Table 1. To test the effects of the carbon skeleton of the tetrahydropyridine ring, we introduced an oxygen atom (**15**), but this showed lower inhibition, suggesting a relatively hydrophobic contact with the enzyme at this position. We installed a methoxy substituent on the benzene ring of the tetrahydroquinoline skeleton (**16**), but this almost completely abrogated inhibitory activity. Finally, we repositioned the cyclopropylamide substituent by one atom in the tetrahydroisoquinoline derivative **17**, but this also greatly lowered activity. These data establish that the tetrahydroquinoline skeleton is quite important to the inhibitory activity of active compounds **1** and **7**.

We proceeded to test variations of Fragment C, the sulfonamide linker between the tetrahydroquinoline and the aromatic substituent, preparing diverse linkers in compounds **18–21** (Table 3). *N*-Hydroxy and *N*-methyl groups combined with sulfonamide (**18** and **19**) were tested, but they showed lower inhibition activity than unsubstituted sulfonamide **7** (Table 1). This suggests possible polar interactions in the enzyme with the N–H group. Amide and urea analogues **20** and **21** were synthesized and assayed but showed almost no activity. Therefore, the simple “N–H” benzenesulfonamide structure in Fragment C was selected as most appropriate for further studies.

We next investigated the effects of variations of Fragments D and E of the core structure (Table 4). Ethyl sulfonamide derivative **22** proved to be quite poorly active, suggesting the importance of the aromatic framework. Subsequently, a variety of substituents on the

benzene ring were prepared and assayed with OGG1 and the OGR1 probe. Compound **23**, carrying a 4-propylphenyl substituent, showed inhibition activity nearly the same as that of compound **7** possessing 4-ethoxyphenyl group. Compound **24**, which extends the alkyl chain compared with **7** was tested, but this did not improve activity. The analogues possessing nitrogen and sulfur atoms on the benzene ring were tested, but the heteroatoms had slightly deleterious effects on activity (**25** and **26**). The disubstituted compound **27** showed lower inhibition activity than monosubstituted compound **7**. Unexpectedly, testing of compound **28** which has a diphenyl ether group, showed almost the same activity as compound **7** with its ethoxy group. Therefore, we designed and synthesized the dibenzofuranyl analogue **29** and biphenyl analogue **30**, which are rigidified variants of the diphenyl ether compound. Interestingly, compound **29** showed low activity, but compound **30** had more potent activity than either diphenyl ether **28** or ethoxyphenyl compound **7**. Cyclohexylphenyl derivative **31** showed almost the same activity as biphenyl derivative **30**, but a more polar morpholinophenyl substituent (**32**) had a poor effect on the activity. Pyrazolylphenyl and pyridylphenyl analogues (**33** and **34**) containing a polar nitrogen atom were subjected to the assay. We found that compound **34** gave slightly stronger inhibitory activity than biphenyl compound **30**, displaying ca. 60% inhibition of activity at 200 nM.

Finally, we explored structural effects of the substituents on the terminal aromatic ring of lead compound **30**, synthesizing compounds **35–39** (Table 5). When 4-trifluoromethyl was used as an electron-withdrawing group and 4-methoxy as an electron-donating group were compared (**35** and **36**), the more polar and electron-donating group (4-methoxy) assisted the activity more than the electron-withdrawing group. Moving this substituent's position, the 3-methoxy compound **37** resulted in more potent activity than the 4-substituted compound **36**. Therefore, varied substituents at the *meta*-position of this benzene ring were examined. Compound **38** (with a dimethylamino group) and compound **39** (possessing an acetylamino group) were synthesized and assayed. Compound **38** showed activity similar to that of methoxy compound **37**, but the acetamide **39** showed higher activity than either of these, with inhibition activity of about 50% at 200 nM. When we investigated compounds **40** and **41**, which have reverse amide structure, these compounds showed yet higher activity, and compound **41** (SU0268), possessing a *meta*-carboxamide group, had the strongest inhibitory activity of all compounds in the study.

Synthesis of SU0268 (Inhibitor **41**)

The synthesis of tetrahydroquinoline biphenyl sulfonamide derivative **41**, the most potent OGG1 inhibitor in this study, is depicted in Scheme 1. We commenced the preparation of **41** with acylation of the commercially available tetrahydroquinoline **42** with cyclopropylcarbonyl chloride in the presence of *N,N*-diisopropylethylamine.⁴¹ Subsequent 1 atm hydrogenation of the acylated intermediate in the presence of palladium on carbon gave the amine **43** in 77%.⁴¹ Sulfonylation of **43** with 4-bromobenzenesulfonyl chloride afforded the sulfonamide **44** in excellent yield.⁴² Construction of the biphenyl structure using Suzuki–Miyaura coupling in the last step with 3-aminocarbonylphenyl boronic acid in the presence of tetrakis-(triphenylphosphine)palladium(0) gave the desired **41** in 83%.⁴³ The intermediates **43** and **44** were obtained by crystallization, and this protocol required only one silica column chromatography purification step to isolate **41**.

Properties of Selected Inhibitors

The properties of selected inhibitors were examined further. Titration curves and IC_{50} values of the hit compound **1**, selected key intermediate inhibitors (**7**, **28**, **34**, and **39**), and optimized compound **41** were determined using the initial rates method (Figure 3 and Table 6). The IC_{50} of the hit compound **1** was $1.7 \mu M$. The IC_{50} values of compounds **7** and **28**, with modified substituents on tetrahydroquinoline and benzenesulfonamide, were slightly more potent than that of **1**. Compounds **34** (with pyridine ring) and **39** (with acetylamino group) had considerably higher activity than the lead compound **1**, with IC_{50} values below $0.3 \mu M$. The IC_{50} value of the optimized compound **41** was $0.059 \mu M$. We calculated hydrophobicity (cLogP) and topological polar surface area (tPSA) values for the compounds (Table 6) as useful measures of potentially bioactive compounds.^{44,45} The values of cLogP of compounds **7** and **28** were higher than for the hit compound **1**, but their tPSA values were almost the same. Our potent inhibitors **34**, **39**, and **41** showed moderate cLogP values (3.3–3.8), and their tPSA values were 79–110, which also fall in the generally accepted range for potential bioactivity.^{44,45}

Surface plasmon resonance (SPR) was used to validate the series and confirm the compounds acted through OGG1. Compound **41** was titrated against biotinylated OGG1 previously captured on a SA streptavidin sensor chip (Figure 4). Sensorgram plots showed a saturable dose response consistent with specific binding to OGG1. The data, fit against 1:1 kinetic and steady-state models, gave K_d 's consistent with IC_{50} of the biochemical assay.

To investigate the effects of SU0268 on inhibition of OGG1, we measured Michaelis–Menten parameters in the absence and presence of this compound at 200 nM and $2 \mu M$. A Lineweaver–Burk plot was obtained from Michaelis–Menten curves (Figure 5), and K_m and k_{cat} values were calculated (Table 7). Measuring the effect of the inhibitor showed an increase in K_m and little or no effect on k_{cat} . These results indicate that inhibitor **41** acts as a competitive inhibitor against OGG1.⁴⁶

We further investigated the interactions of abasic DNA (without 8-OG) and OGG1 probe (with 8-OG) with inhibitor **41** (Table 8). Abasic DNA has C instead of tCo and was introduced abasic site mimic (X) instead of 8-OG. If the interaction is observed, melting temperature (T_m) would be changed significantly.⁴⁷ In both cases of abasic DNA and OGG1 probe, no measurable changes of T_m were observed, providing evidence that inhibitor SU0268 does not bind DNA and thus interacts with OGG1 specifically rather than its substrate.

The selectivity of inhibitor SU0268 (**41**) was investigated by testing it against other repair enzymes that process either 8-OG nucleotides, bind nucleotides or DNA, or employ base excision mechanisms. MTH1 (human MutT Homolog 1),⁴⁹ dUTPase (deoxyuridine 5'-triphosphate nucleotidohydrolase),⁵⁰ NUDT16 (nucleoside diphosphate-linked moiety X-type motif 16),⁵¹ hABH2 (human AlkB Homolog 2),⁵² hABH3 (human AlkB Homolog 3),⁵³ and SMUG1 (single-strand selective monofunctional uracil DNA glycosylase)⁵⁴ were assayed with the compound at $20 \mu M$ (Figure 6). The activities against all enzymes of compound **41** were low or negligible, thus establishing high selectivity of compound **41** to OGG1.

The toxicity of compound **41** was tested in two human cell lines (HEK293T and HeLa) by the MTT assay (Figure 7). The compound showed little or no cytotoxicity in both cell lines at concentrations 10 nM to 10 μ M and exhibited moderate apparent toxicity only at the highest 100 μ M concentration (30–50% toxicity). Thus, the compound is not significantly toxic at concentrations well above its IC₅₀.

Next, we examined the membrane permeability of SU0268 (**41**) using the Caco-2 (clone C2BBel) assay, measuring permeability in both the apical-to-basolateral and basolateral-to-apical directions (Table 9). Recoveries based on the apical side (A-to-B) and basolateral side (B-to-A) were 65% and 79%, showing that the permeability of compound **41** is satisfactory.

Finally, we carried out tests of the ability of SU0268 to engage OGG1 and inhibit its activity in a human cell line. Since the cellular role of the enzyme is to remove 8-oxoG from DNA, inhibition of the enzyme is expected to measurably increase levels of the damaged base in DNA. To evaluate this possibility, we incubated cells for 24 h with or without SU0268 at 0.5 μ M and used LC/MS-MS methods to measure levels of this damaged base in DNA isolated from the cells. The results (see Figure 8a) confirm that the drug increases levels of 8-oxodG in DNA by 25% after only 24 h relative to the mock-treated controls. The amount of increase is similar to or greater than that provided by a positive control of oxidatively damaging conditions. We also employed a fluorescence probe of OGG-1 activity³⁹ to directly test the ability of SU0268 to inhibit OGG1 activity in HeLa and MCF-7 tumor cell lysates lines, which have relatively low and high OGG1 activities, respectively. Results of the assay are shown in Figure 8b; the data confirm the inhibition of OGG1 activity at 0.5 μ M of the compound, amounting to 64% and 95% inhibition of probe signal in the two lysates as measured at 20 h. Thus, the collective data provide evidence that SU0268 (**41**) can be active at the intended cellular target, both in lysates and in living cells.

A previous report described the discovery of hydrazine and hydrazone inhibitors of OGG1 using a DNA strand cleavage assay.³⁵ We independently confirmed that two of the most potent of those compounds are indeed inhibitors of the enzyme *in vitro* (Supporting Information, Figure S1) using our base excision (OGR1) assay. The results show that the new compound SU0268 (**41**) (IC₅₀ = 0.059 μ M) is more potent than those prior compounds as measured by the initial rates method. Notably, the prior compounds exhibit apparently delayed kinetics of inhibition, which suggests the possibility that the enzyme may first excise the 8-OG base before the compounds can be active (see Figure S1). In contrast, as measured by the OGR1 assay, the current inhibitor SU0268 binds directly to OGG1 enzyme, even in the absence of DNA, and directly inhibits base excision, the first step of repair of this lesion by this enzyme.

In summary, we have developed highly potent and selective OGG1 inhibitor SU0268, which has an acyl tetrahydroquinoline sulfonamide skeleton. The structure–activity relationships of the compounds were outlined by synthesizing a broad range of analogues. Synthesis of the optimized OGG1 inhibitor is quite straightforward from commercially available starting materials. The compound shows good membrane permeability and no cytotoxicity in HEK293T and HeLa cells at active concentrations and demonstrates activity in inhibiting the

enzyme in human cell lines. Further studies are being directed to applications of this inhibitor in varied cellular and animal models of multiple diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HTS of 25,975 compounds at a screening concentration of 20 μ M

8-point validation assay for 360 compounds >35% inhibition at 20 μ M
22 confirmed inhibitors

Validate hit compound 1

PubChem CID: 7517554

Primary screen: 91% inhibition

Absolute IC_{50} = 2 μ M

Figure 2.

Discovery of lead compound **1** and strategy for the lead optimization. Following the HTS compounds were validated using eight concentration points ranging from 100 to 0.03 μ M. Absolute IC_{50} is the concentration for which the concentration response curve crosses 50% inhibition.

Figure 3.
Titration curves of selected OGG1 inhibitors in this study.

Figure 4. SU0268 (**41**) specifically binds OGG1. 3000 RU (resonance units) of recombinant biotinylated OGG1 was immobilized onto streptavidin sensor chip SA, and experiments were performed on a Biacore 8K with a 1:3 dilution series of inhibitor **41** (highest concentration 4 μ M) (a) Sensorgram plots of inhibitor **41**. (b) Steady-state K_D determination of compound **41**. (c) Kinetic and binding constants.

Figure 5.
Lineweaver–Burk plots of OGG1 activity without inhibitor and with inhibitor **41**.

Figure 6.
Selectivity of compound **41**, tested by assaying against varied repair enzymes at 20 μ M.

Figure 7.
Toxicity of 41 (SU0268) by MTT assay with HEK293T and HeLa cell lines.

Figure 8. Inhibition of cellular OGG1 activity by **41** (SU0268). (a) Incubation with 0.5 μ M SU0268 increases the number of 8-oxodG lesions in HeLa cell DNA. (b) SU0268 (0.5 μ M) inhibits OGG1 activity as measured by fluorescence probe (pOGR1)³⁹ in HeLa and MCF-7 cell lysates.

Scheme 1.
Synthesis of the OGG1 Inhibitor SU0268 (41)

Table 1Examination of Substituents R¹ and Their Effects on OGG1 Activity (Fragment A)

Cmpd	R ¹	Enzyme activity, % of control at 20 μ M ^a	2 μ M ^{a,b}	20 μ M ^{a,b}
1		4.7 $\pm$ 3.0	47 $\pm$ 3.5	74
2		3.7 $\pm$ 0.83	51 $\pm$ 1.4	n.t.
3		79	100	n.t.
4		51	93	n.t.
5		52	96	n.t.
6		100	n.t.	n.t.
7		5.8 $\pm$ 1.9	41 $\pm$ 1.7	82 $\pm$ 12
8		33	69	n.t.
9		92	96	n.t.
10		77	87	n.t.
11		83	100	n.t.

^a % of control values are ratio of enzyme activity to control (no compound) based on slopes of initial rate (first 12 min of assay).

^b n.t., not tested.

Table 2Examination of Core Structures R² and Their Effects on OGG1 Activity (Fragment B)

Cmpd	R ²	Enzyme activity, % of control at 20 μM ^a	2 μM ^{a,b}	200nM ^{a,b}
1		4.7 ± 3.0	47 ± 3.5	74
7		5.8 ± 1.9	4.1 ± 1.7	82 ± 12
12		70	96	n.t.
13		100	95	n.t.
14		35	76	n.t.
15		41	90	n.t.
16		93	100	n.t.
17		88	94	n.t.

^a% of control values are ratio of enzyme activity to control (no compound) based on slopes of initial rate (first 12 min of assay).^bn.t., not tested.

Table 3Examination of Linkers R³ and Their OGG1 Inhibition Activity (Fragment C)

Cmpd	R ³	Enzyme activity, % of control at 20 μ M ^a	2 μ M ^{a,b}	200 nM ^{a,b}
1		4.7 $\pm$ 3.0	47 $\pm$ 3.5	74
7		5.8 $\pm$ 1.9	41 $\pm$ 1.7	82 $\pm$ 12
18		21	79	100
19		79	84	n.t.
20		100	100	n.t.
21		100	100	n.t.

^a% of control values are ratio of enzyme activity to control (no compound) based on slopes of initial rate (first 12 min of assay).^b n.t., not tested.

Table 4Examination of Substituents R⁴ and Their Effects on OGG1 Activity (Fragments D and E)

Cmpd	R ⁴	Enzyme activity, % of control at 20 μ M ^a	2 μ M ^a	200 nM ^{a,b}
1		4.7 $\pm$ 3.0	47 $\pm$ 3.5	74
7		5.8 $\pm$ 1.9	41 $\pm$ 1.7	82 $\pm$ 12
22^c		74	94	n.t.
23		14	35	74
24		n.t.	19	95
25		10	58	100
26		3.6	53	75
27		22	71	n.t.
28		8.3	41 $\pm$ 2.0	100
29		71	82	n.t.
30		n.t.	29 $\pm$ 7.0	90 $\pm$ 6.2
31		n.t.	25	87
32		n.t.	76	84
33		14 $\pm$ 3.2	45 $\pm$ 1.3	81
34		2.0	23 $\pm$ 1.5	61 $\pm$ 1.3

^a % of control values are ratio of enzyme activity to control (no compound) based on slopes of initial rate (first 12 min of assay).

^b n.t., not tested.

^c Acyl group of nitrogen atom on tetrahydroquinoline skeleton is propionyl group.

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Table 5Examination of Substituents R⁵ and Their OGG1 Inhibition Activity (Fragment E)

Cmpd	R ⁵	Enzyme activity, % of control at 20 μ M ^{a,b}	2 μ M ^{a,b}	200 μ M ^a
1		4.7 $\pm$ 3.0	47 $\pm$ 3.5	74
7		5.8 $\pm$ 1.9	41 $\pm$ 1.7	82 $\pm$ 12
35		n.t.	72	97
36		n.t.	31	79
37		n.t.	13	67
38		n.t.	n.t.	70
39		n.t.	12 $\pm$ 2.0	53 $\pm$ 4.9
40		n.t.	n.t.	48
41		n.t.	4.8	39 $\pm$ 2.5

^a % of control values are ratio of enzyme activity to control (no compound) based on slopes of initial rate (first 12 min of assay).^b n.t., not tested.

Table 6IC₅₀, cLogP, and tPSA Values of OGG1 Inhibitors

compd	IC ₅₀ (μ M)	cLogP ^a	tPSA ^a
1	1.7	2.5	76
7	1.1	3.6	76
28	1.5	4.9	76
34	0.27	3.4	79
39	0.25	3.8	96
41	0.059	3.3	110

^aSee SI for calculation methods.

Table 7 K_m and k_{cat} without Inhibitor and with Inhibitor 41

	no inhibitor	200 nM 41	2 μ M 41
K_m (μ M)	1.2	1.8	8.2
k_{cat} (s^{-1})	0.046	0.040	0.057
k_{cat}/K_m ($s^{-1} \mu$ M $^{-1}$)	0.039	0.022	0.0070

Table 8

Thermal Denaturation Studies of OGR1 Probe and Abasic DNA in the Absence ($T_{m(-)}$) and Presence ($T_{m(+)}$) of OGG1 Inhibitor 41

5'- C G C G C C G G A A G C G tCo oG G C A A OGR1 Probe		5'- C G C G C C G G A A G C G C X G C A A Abasic DNA	
DNA	$T_{m(-)}$ (°C)	$T_{m(+)}$ (°C)	T_m (°C)
abasic DNA ^a	71.3 ± 0.63	71.3 ± 0.29	0.0
OGR1 probe ^b	82.2 ± 0.77	82.5 ± 0.25	0.3

^a[DNA] = 1 μ M, [41] = 2 μ M (1% DMSO), NEBuffer.

^b[DNA] = 1 μ M, [41] = 2 μ M (10% DMSO), PBS buffer, and betaine (10 mM).⁴⁸

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Table 9

CACO-2 Assay with the OGG1 Inhibitor 41

direction	recovery (%)	$P_{app} (\times 10^{-6} \text{ cm/s})$			avg	efflux ratio
		R1	R2			
A-to-B	65	14.2	10.8	12.5		6.2
B-to-A	79	63.5	91.3	77.4		