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Dual Inhibitors of 8-Oxoguanine Surveillance by OGG1 and NUDT1

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Abstract

Oxidative damage in DNA is one of the primary sources of mutations in the cell. The activities of repair enzymes 8-oxoguanine DNA glycosylase (OGG1) and human MutT Homologue 1 (NUDT1 or MTH1), which work together to ameliorate this damage, are closely linked to mutagenesis, genotoxicity, cancer, and inflammation. Here we have undertaken the development of small-molecule dual inhibitors of the two enzymes as tools to test the relationships between these pathways and disease. The compounds preserve key structural elements of known inhibitors of the two enzymes, and they were synthesized and assayed with recently developed luminescence assays of the enzymes. Further structural refinement of initial lead molecules yielded compound **5** (SU0383) with $IC_{50}(\text{NUDT1}) = 0.034 \mu\text{M}$ and $IC_{50}(\text{OGG1}) = 0.49 \mu\text{M}$. The compound SU0383 displayed low toxicity in two human cell lines at $10 \mu\text{M}$. Experiments confirm the ability of SU0383 to increase sensitivity of tumor cells to oxidative stress. Dual inhibitors of these two enzymes are expected to be useful in testing multiple hypotheses regarding the roles of 8-oxo-dG in multiple disease states.

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/acscchembio.9b00490.

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

The authors declare no competing financial interest.

Damage to cellular DNA is closely linked to mutagenesis, genotoxicity, and tumorigenesis.¹⁻⁶ 8-Oxoguanine (8-OG) is the most abundant form of oxidative damage to DNA bases and is produced by reactive oxygen species resulting from metabolism or exposure to factors that are driven by oxidative stress.⁷⁻¹⁷ Because mispairing of 8-OG with adenine frequently occurs during DNA replication, the oxidized base is highly mutagenic,¹⁸ and this form of damage may be the greatest single source of mutations in the cell.¹⁵ The existence of 8-OG in DNA can be hazardous to cell growth by leading to DNA double-strand breaks after its excision.¹⁹ This damaged base can arise in DNA both by direct oxidative damage to the nucleic acid and also by polymerase incorporation of 8-oxo-dGTP, which arises from oxidative damage to the cellular nucleotide pool.²⁰

Two cellular enzymes, 8-oxoguanine DNA glycosylase (OGG1) and human MutT Homologue 1 (NUDT1 or MTH1), carry out the majority of surveillance and amelioration of 8-OG in DNA and nucleotides, and both enzymes have shown strong relevance to cancer and human health. The enzymes exist in the nucleus and mitochondria, repairing damage in both subcellular locations.^{21,22} The first of these, OGG1, acts *via* the base excision repair (BER) pathway.²³ The enzyme recognizes 8-OG in double-stranded DNA and cleaves the glycosidic bond, releasing 8-OG as a free base and producing an abasic site in the DNA.²³ Lyase activities of the OGG1 enzyme itself, or the AP lyase enzyme, then further process this abasic site, ultimately leading to strand cleavage.^{19,24,25} Under high oxidative stress, proximity of multiple repair sites in both DNA strands can result in genotoxic double-strand breaks.¹⁹ If the damage is not too frequent, then additional enzymes in the BER pathway can repair the damage, regenerating intact DNA with correctly paired bases.²³

Previous studies have shown strong relationships between OGG1 activity and multiple pathologic conditions, including HNSCC (head and neck squamous cell carcinoma),²⁶ breast cancer,²⁷ lung cancer,²⁸⁻³⁰ inflammation,³¹ and rheumatoid arthritis.³² Mice deficient in OGG1 expression have been shown to have elevated levels of 8-OG in their DNA and increased cellular mutations.^{33,34} Further, 8-OG has been identified as a signaling molecule to modulate activity of several GTPases.³⁵ siRNA-mediated downregulation of OGG1 activity has been shown to decrease lung inflammation in murine allergy models,³¹ associated with downregulation of proinflammatory signaling pathways, and the enzyme has been suggested as a therapeutic target for control of inflammatory responses. Very recently, small molecule inhibitors of OGG1 were described,³⁶⁻³⁸ and one inhibitor was shown to decrease inflammatory responses in a mouse model.³⁸

8-OG enters DNA not only from direct oxidative damage of the biopolymer but also from polymerase incorporation of the damaged nucleotide 8-oxo-dGTP. The second enzyme addressed here, NUDT1, functions as a phosphohydrolase of 8-oxo-dGTP, generating polymerase-inactive 8-oxo-dGMP and pyrophosphate.³⁹ The enzyme is necessary to cleanse this damage in the nucleotide pool, which can contribute to cellular mutations.⁴⁰ While MTH1 activity is needed for suppressing mutations in normal cells, it is not essential for cell viability.⁴⁰ Mice lacking the *NUDT1* gene show a similar mutagenic phenotype as with OGG1 knockouts, with elevated 8-OG in DNA and increased levels of mutations.^{40,41} However, cancer cells can become dependent on NUDT1 to maintain their rapid growth.⁴² Tumors possessing mutations in the RAS proto-oncogenes commonly display elevated levels

of reactive oxygen species (ROS) with damage including 8-OG.⁴³⁻⁴⁵ Thus, tumor cells often express high NUDT1 levels to act against the toxicity of elevated ROS in these rapidly growing cells.^{46,47} As a result, MTH1 inhibition as a potential anticancer strategy has been under intense study recently,⁴⁸⁻⁵³ and clinical trials of an inhibitor are underway.⁵⁴ Studies by Helleday and co-workers have documented inhibition of tumor cell proliferation by NUDT1 inhibitors in certain tumor cell lines. In contrast, multiple studies with different NUDT1 inhibitors have shown a lack of activity in suppressing tumor cell growth.⁵⁰⁻⁵² The lack of effect in some tumor cell lines may be explained in some cases by use of cell line models that do not have high levels of NUDT1 activity and the existence of cellular enzyme activities that may compensate for low NUDT1 activity.⁵⁵ Until recently⁵⁶ it has been difficult to measure this enzymatic activity in cell and tissue lysates, making choice of appropriate cell lines difficult. One candidate enzyme that may compensate for low NUDT1 activity is OGG1, which can repair 8-OG in DNA after being incorporated from the cellular nucleotide pool.

Dual inhibition of NUDT1 and OGG1 would enable the testing of the interdependence of these two repair pathways, by downregulating the two primary enzymes that limit the presence of 8-OG in DNA. There are multiple motivations for the development of dual inhibitors of these enzymes. First is *RAS*-dependent tumor growth; if inhibition of NUDT1 alone is not sufficient to generate useful levels of tumor suppression, and OGG1 rescues cells from the added 8-OG burden, then dual inhibition is expected to maximize levels of the damaged base in DNA. This might more effectively sensitize cells to oxidative stress or potentially cause suppression of growth *via* hypermutation.⁵⁷ A second motivation is to maximize 8-OG and mutagenesis of cellular DNA in tumors, resulting in increased neoantigen load. Increased levels of mutations and impaired DNA repair have been strongly correlated to improved response of cancer patients to checkpoint immunotherapy.⁵⁸ A third reason to inhibit both enzymes is to further reduce the amount of 8-OG released from DNA, as well as OGG1-DNA binding, during inflammatory responses;³¹ dual inhibitors thus could be useful in models of inflammation. Although individual inhibitors of NUDT1 and OGG1 could in principle be used in combination, a single-agent dual inhibitor molecule would simplify cellular and animal studies by avoiding some complexities of polypharmacology, such as differential solubility, potency, differential half-lives, and additive off-target effects.

To target the two enzymes together, we first considered known inhibitors for each enzyme individually. Potent NUDT1 inhibitors with varied chemical structures have been developed,⁴⁸⁻⁵³ and we recently developed the potent and selective OGG1 inhibitor SU0268³⁷ (Figure 1). This compound inhibits the base excision step of OGG1 (distinct from the lyase step) and was discovered with the aid of a fluorogenic probe (OGR1, see Figure 3). Most NUDT1 inhibitors contain an aminopyrimidine core that is known to engage the active site 8-OG-binding residues, along with hydrophobic aromatic residues to add binding affinity and specificity. Crystal structures of NUDT1 with inhibitors bound in the active site are known,⁴⁸⁻⁵³ which can be helpful in designing new variants. The OGG1 inhibitor SU0268 contains an acyl-tetrahydroquinoline core with an arenesulfonyl chain that adds affinity. Although structures of OGG1 bound to DNA are reported,⁵⁹⁻⁶² no structural studies of this bound inhibitor are yet available; therefore, we relied on structure–activity relationships³⁷ to guide dual inhibitor designs.

Here, we describe the design and development of the first small-molecule OGG1/NUDT1 dual inhibitors using recently developed fluorogenic and luminogenic enzyme-specific assays,^{56,63} leading to the identification of a tetrahydroquinoline scaffold possessing a 2-aminopyrimidine moiety with significant inhibitory activity against both enzymes. Dual inhibitor **5** (SU0383) is found to be an effective and cell-active inhibitor with low toxicity, suggesting its future utility as a probe for testing hypotheses regarding oxidative damage, mutagenesis, tumor cell growth, and inflammation.

RESULTS AND DISCUSSION

Dual Inhibitor and Assay Design.

Our design of a dual NUDT1/OGG1 inhibitor started with preserving and combining key pharmacophores of prior inhibitors of the two enzymes. A recent study demonstrated that a tetrahydroquinoline biphenylsulfonamide structure confers potent OGG1 inhibition activity.³⁷ In addition, varied substituents at the 3-position on the biphenyl structure maintained activity. These results indicate that the OGG1 binding site of such inhibitors may have steric room to accept other types of substituents. On the other hand, representative NUDT1 inhibitors, TH287, TH086, IACS-4619, and IACS-4759 (Figure 1), possess 2-aminopyrimidine skeletons that interact with asparagine and aspartic acid in the NUDT1 active site pocket.^{48,50} This latter enzyme's binding site accepts diverse substituents at the 6-position on the 2-aminopyrimidine moiety, including varied hydrophobic substituents that enhance the inhibition activity.^{50,53} For example, the compound AZ13792138 with a large hydrophobic cyclic moiety showed highly potent NUDT1 inhibition activity.⁵¹ Our conceptual designs for dual inhibitors make use of the above information and combine these chief pharmacophores in two ways (Figure 2a): either by overlapping/merging the structures, using the biphenyl motif of SU0268 as a replacement for the hydrophobic motif of NUDT1 inhibitors, or by a combination strategy, linking basic inhibitor cores to one another. Our initial three structures aimed at testing these strategies (compounds **1–3**) are shown in Figure 2b.

For measuring activities of the two enzymes, we recently developed the ATP-releasing guanine-oxidized probe (ARGO) that acts as a luminescence reporter of NUDT1 activity recognizing 8-oxo-dGTP⁵⁶ and the fluorogenic probe OGR1 which can quantify the 8-OG base excision activity of OGG1⁶³ (Figure 3). The two luminescence probes were employed to conveniently measure inhibition activity of candidate dual inhibitors for *in vitro* assays.

Synthesis and Inhibitory Properties.

Compounds **1–3** were synthesized for initial testing as dual inhibitors; full synthesis and characterization details are given in the Supporting Information. Compound **1** incorporates the diaminopyrimidine motif of NUDT1 inhibitor TH287⁴⁹ and replaces its aryl hydrophobic substituent with part of the biphenyl substituent of OGG1 inhibitor SU0268, making a relatively small merged design (Figure 2b). The somewhat larger compound **2** uses a similar merged design but preserves both aryl rings of the biphenyl moiety of SU0268 and attaches the pyrimidine moiety *via* a meta orientation reminiscent of SU0268. Compound **3** adopts the linking strategy and starts with NUDT1 inhibitor IACS-4759,⁵⁰ which contains a

hydroxyalkyl linker that we hypothesized might be adopted as a linker moiety. This is connected to SU0268 *via* its biphenylamide group.

The inhibitory activities of compounds **1–3** with each enzyme were investigated with the luminescence probe assays (Figure 3). Compounds were initially measured over a three-log concentration range in the NUDT1 assay (200 nM–2 nM) and a two-log concentration range in the OGG1 assay (2 μ M and 200 nM) to differentiate weak inhibitors from stronger ones. Compound **1** showed no activity at 200 nM in the NUDT1 assay (Figure S2) but displayed potent inhibitory activity at 200 nM in OGG1 assay. The larger compound **2**, which possesses a 2-aminopyrimidine moiety at the 3-position on the biphenyl group of SU0268, was found to be a strong NUDT1 inhibitor, with ca. 60% inhibition activity at 2 nM. Compound **2** acted as an OGG1 inhibitor as well, with inhibitory activity against OGG1 of ca. 70% at 200 nM. These results establish that choice of the linker between NUDT1 and OGG1 inhibitor moieties is critical for promoting dual activity. Compound **3**, which connects SU0268 with the 2-aminopyrimidine moiety through its 2,2-dimethylpropylene linker, also retained both NUDT1 and OGG1 inhibitory activity (albeit with somewhat lowered potency for the latter), establishing that use of a linking strategy was more effective than the merged designs.

Because its straightforward linker-based design simplifies the construction of analogues, we then focused on variants of compound **3** to improve its properties as a practical probe molecule (Figure 4). To reduce hydrophobicity and molecular weight, compound **4**, which omits the dimethyl substituents, and chain-shortened compound **5** were synthesized and assayed (Figure S1). Compound **4** displayed ca. 50% inhibitory activity at 20 nM in the NUDT1 assay and more potent activity than compound **3** in the OGG1 assay (Figure 4). Dual assay of compound **5** revealed further improved activity against both enzymes. Given its more favorable hydrophobicity profile, lower molecular weight, and dual inhibitory activity, compound **5** (SU0383) was selected for further study in comparison with related analogs **3** and **4**.

The synthetic approach used for preparation of dual inhibitors **3–5** is shown in Scheme 1. We commenced the preparation of 4-bromobenzenesulfonamide derivative **8** by acylation of the commercially available tetrahydroquinoline **6** and followed this by addition of a bromoarylsulfonyl group.³⁷ Suzuki-Miyaura coupling of **8** with 3-ethoxycarbonyl-phenylboronic acid in the presence of tetrakis(triphenylphosphine)-palladium(0) and subsequent hydrolysis gave carboxylic acid **10**. Condensation of **10** with amino alcohols using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide and 1-hydroxybenzotriazole afforded the amide bond on the biphenyl structure of tetrahydroquinoline sulfonamide skeleton in excellent yield. As the last step, nucleophilic aromatic substitution of **11–13** using 4-chloro-5-methylpyrimidin-2-amine in the presence of sodium hydride gave the desired compounds **3–5** in moderate yield.

Modeling Structures of OGG1 and NUDT1 Complexes.

To better understand the potential interactions and structures of the complexes of the best-performing dual inhibitor with these two target enzymes, we carried out docking studies,

allowing the opposite ends of the linked compound SU0383 to interact *in silico* with the two human repair enzymes in separate experiments. Starting structures were obtained from recently published structures of each of the two separate proteins with small molecule inhibitors.^{38,50} Figures 5 and S4 show plausible structures of complexes of SU0383 with the two enzymes. In the OGG1 complex, modeled in analogy to the reported complex of experimental drug TH5675 with murine OGG1,³⁸ the tetrahydroquinoline pharmacophore of the compound is well accommodated in the same pocket as the published ligand. The linker and aminopyrimidine heterocycle of SU0383, which are not targeted to this enzyme, are extended near the outside surface of the protein and make fewer interactions. For the NUDT1 complex, the aminopyrimidine motif of SU0383 can bind similarly to the modeled complex of the same motif of the drug IACS-4759;⁵⁰ the linker and remainder of the compound project toward the outside of the enzyme pocket with little apparent structural disruption of the protein. Initial studies of merged variant **1** showed no ability of the aminopyrimidine motif to engage in this way (data not shown) due to the rigidified structure. Overall, the *in silico* docking studies reveal plausible structures for the two complexes of SU0383 and help explain why the linker strategy (e.g., compounds **3–5**) was more effective than the “merged” strategy (compounds **1** and **2**) in our inhibition studies.

Biochemical and Cellular Activities.

Properties of the dual inhibitors were further examined. Titration curves and IC₅₀ values of the initially tested compounds **2** and **3**, along with dual inhibitors **4** and **5**, were determined toward both NUDT1 and OGG1 inhibition activity (Figure 6 and Table 1). The IC₅₀ values of the compound **2**, with aryl structure combined with the tetrahydroquinoline sulfonamide skeleton, were 0.0093 μM (NUDT1) and 0.62 μM (OGG1). The IC₅₀ values of linked compound **3** were 0.0060 μM (NUDT1) and 2.7 μM (OGG1). Compound **4**, lacking the 2,2-dimethyl group on the linker, had lower activity than **3** in NUDT1 inhibition but improved activity in OGG1 inhibition. The optimized compound **5** displayed better-balanced IC₅₀ values of 0.034 μM (NUDT1) and 0.49 μM (OGG1).

Compound SU0383 (**5**) was studied in further detail as a potential cellular probe. Toxicity was evaluated with two human cell lines (MCF-7 and HeLa), and SU0383 displayed little or no measurable toxicity at concentrations up to 10 μM (Figure 7). We examined the membrane permeability of SU0383 using the Caco-2 (clone C2BBel) assay (Table 2). Recoveries based on the apical side (A-to-B) and basolateral side (B-to-A) were 40% and 43%, respectively, showing that the permeability of compound **5** falls within the acceptable range for bioactive small molecules.⁶⁴ The measured efflux ratio of 5.1 suggests active efflux of the compound and implies that use of elevated concentrations may be required to achieve effective intracellular levels of the dual inhibitor. The efflux may also have the effect of lowering the apparent toxicity.

Next, we proceeded to measure the ability of SU0383 to engage the two native enzymes in cellular media. To implement this, thermal shift analysis (CETSA) studies were carried out with intact MCF7 cells in the presence of SU0383 at 10 μM , employing antibodies specific for the two target proteins. The results show that SU0383 engages NUDT1, showing marked thermal stabilization of the protein relative to GADPH control (Figure S5). The data for

OGG1 are inconclusive due to a combination of low expression levels and high inherent thermal stability of the protein. To further test the interaction of SU0383 with native OGG1 from the cells, we used the pOGR1 probe to carry out measurements of OGG1 activity in MCF7 lysate in the absence and presence of the dual inhibitor (Figure S6). The data show clear inhibition by SU0383 at 10 μ M, reducing signals and enzyme activities to levels similar to those achieved with the more potent single inhibitor SU0268.

The enzymes OGG1 and NUDT1 serve to protect cells from oxidative damage and may enhance tumor cells' ability to withstand high levels of reactive oxygen species. We proceeded with an additional test of cellular activity of SU0383, by examining whether SU0383 can affect cellular responses to oxidative stress. We exposed breast cancer-derived MCF7 cells (which express high levels of NUDT1 and OGG1 activity) to peroxide and measured cell viability after 12 h. Results showed that SU0383 sensitized cells to this oxidative stress, increasing the effective toxicity of this reactive oxygen species ($p = 0.009$; Figure 8). This is consistent with the known effects of knockout animal models of these two enzymes,⁴¹ which display elevated levels of accumulated DNA damage and mutations, and also consistent with the expectation that cellular repair of oxidative damage to deoxyguanine residues can prevent cytotoxicity in the presence of high levels of reactive oxygen species. Interestingly, the compound was more effective than the monofunctional OGG1 inhibitor SU0268, implying that of these two pathways, NUDT1 plays a greater role in detoxification against this reactive oxygen species than does OGG1. More work will be needed to study the differential roles of these two enzymes in varied cell lines; however, the results confirm that SU0383 can function to alter cellular responses.

In summary, we have developed potent OGG1/NUDT1 first-in-class dual inhibitor SU0383, which has an acyl tetrahydroquinoline sulfonamide skeleton linked with 2-aminopyrimidine at its biphenyl moiety. Compound SU0383 and its analogues are synthesized in a straightforward fashion from commercially available starting materials. The compound shows acceptable membrane permeability and low cytotoxicity in MCF-7 and HeLa cells at active concentrations and demonstrates activity in sensitizing tumor cells to oxidative stress. Further studies are being directed to applications of this dual inhibitor as a probe in varied cellular and animal models of multiple disease states.

MATERIALS AND METHODS

General.

¹H NMR spectra were recorded on Varian Inova 300 (300 MHz) spectrometers. Low-resolution mass spectra were measured on an ESI (Electro Spray Ionization) by the ACQUITY UPLC (Waters). High-resolution mass spectra (HRMS) were measured on an ESI by micrOTOF-Q II (Bruker). Ultraviolet spectra were measured on a Varian Cary 300. Fluorescence intensities were measured on a Fluoroskan Ascent Microplate Fluorometer. The OGR1 probe was synthesized with an Applied Biosystems 394 DNA/RNA synthesizer as described.⁶³ Analytical TLC 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).

Synthesis and Characterization of Inhibitor Candidates.

Details of synthesis and characterization are given in the Supporting Information.

OGG1 Inhibition 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. Synthesized compounds (1% DMSO) 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, the OGR1 probe⁶³ (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). Fluorescence data were obtained for each compound (Supplementary Figure S1), and the slope of the initial rate (12 min) was calculated. OGG1 inhibition activity is shown as % of control values that are ratios of enzyme activity to control (no compound) based on the initial rate (Supplementary Figure S2). For determination of IC₅₀ values, equations for curve fitting (Rodbard Equation) were obtained from ImageJ software with data of each concentration (0.002 μ M–20 μ M).

NUDT1 Inhibition Assay.

MTH1 (NUDT1) was purchased from Abcam, PNK buffer was purchased from New England Biolabs, and Kinase-Glo was purchased from Promega. Synthesized compounds (DMSO solution, 1 μ L), MTH1 (500 nM, 0.8 μ L), and PNK buffer (1X) solution of the ARGO probe⁵⁶ (40 μ M, 20 μ L) were mixed and incubated at 30 °C (30 min) in a white 384-well plate (21.8 μ L reaction volume). After that, 5 μ L of this reaction solution was mixed with 20 μ L of Kinase-Glo. Immediately, luminescence was measured on a Thermo Fluoroskan Ascent FL fluorescence plate reader. Luminescence data were obtained for each compound (Supplementary Figure S1), and ratios of enzyme activity to control (no compound) based on maximum point of initial rate (within first 9 min of assay) were used for MTH1 inhibition activity (Supplementary Figure S2). The titration curves were plotted (0.2 nM–20 μ M), and IC₅₀ values were determined using Graphpad Prism by fitting to log(inhibitor) vs response model ($Y = \text{bottom} + (\text{top} - \text{bottom}) / (1 + 10^{((X - \text{LogIC}_{50}))})$).

Molecular Docking Studies.

Docking studies were performed using the Maestro 12.0 (Release 2019–2) software package (Schrödinger, LLC, New York, 2019). The protein structures were extracted from the following PDB entries: 3ZR0 (MTH1/8-O-G), 6G3Y (mOGG1/TH5675), 5KO9 (hOGG1). The binding pockets were determined by replacing the initial ligands. Conformational refinement was performed by the OPLS3e force field as implemented in the software. Solvent molecules as well as ions contained in the crystal structures were removed prior to the docking. In the case of the mOGG1 model, the chains were separately extracted from the PDB entry. Figure 5 shows the results of the docking studies for chain A. During the docking the extra precision (XP) mode was used, and flexible docking of the ligand was allowed. The visualization of the results was done within the Maestro software. For the alignment of the OGG1 structures the software Chimera (UCSF Chimera 1.12) was used and done by the implemented MatchMaker tool.

Toxicity via MTT Assay.

MCF-7 and HeLa cells (1.2×10^4 cells per well) were seeded to a 96-well plate in supplemented DMEM culture medium (10% FBS, 100U penicillin/streptomycin) and incubated for 16 h at 37 °C, 95% humidity, and 5% CO₂. These were incubated in 100 μ L of the fresh medium at a concentration of 10 μ M, 1 μ M, 100 nM, and 10 nM of the compound **5** for 24 h at 37 °C, 95% humidity, and 5% CO₂, in supplemented DMEM culture medium. After the incubation period, 10 μ L of the MTT labeling reagent (final concentration 0.5 mg mL⁻¹) was added to each well and incubated at 37 °C, 95% humidity, and 5% CO₂. 100 μ L of the solubilization solution was added into each well after 4 h and incubated for the next 20 h at 37 °C, 95% humidity, and 5% CO₂. Absorbance of the samples was measured by using a microplate reader (Tecan Infinite M1000) at 550 and 650 nm. Compound **5** was provided in concentration of 2 mM in DMSO, so in all samples (10 μ M, 1 μ M, 100 nM, 10 nM) concentration of DMSO was at the level of 1%. The controls were prepared by incubating cells in the supplemented DMEM containing 1% DMSO. The experiment was prepared in five replicates.

Oxidative Stress Studies.

MCF-7 cells (1.2×10^4 per well) were seeded to a 96-well plate in supplemented DMEM culture medium (10% FBS, 100U penicillin/streptomycin) and incubated for 16 h at 37 °C, 95% humidity, and 5% CO₂. DMEM was replaced with 100 μ L of the fresh medium at a concentration of 10 μ M of the compound **5** and cell incubated for 4 h at 37 °C, 95% humidity, and 5% CO₂. After the incubation period, fresh supplemented medium containing 10 μ M of the compound **5** and hydrogen peroxide in the concentration range 1–16 mM was added and cell cultured for 20 min. The medium was replaced by supplemented DMEM with 10 μ M of the compound **5** and cell cultured for the next 12 h. The viability of cells was measured as described above, using MTT assay. Ten microliters of the MTT labeling reagent (final concentration 0.5 mg mL⁻¹) was added to each well and incubated at 37 °C, 95% humidity, and 5% CO₂. One hundred microliters of the solubilization solution was added into each well after 4 h and incubated for the next 20 h at 37 °C, 95% humidity, and 5% CO₂. Absorbance of the samples was measured by using a microplate reader (Tecan Infinite M1000) at 550 and 650 nm. Compound **5** was provided in concentration of 2 mM in DMSO, so in all samples (10 μ M, 1 μ M, 100 nM, 10 nM) concentration of DMSO was at the level of 1%. The controls were prepared by incubating cells in the supplemented DMEM containing 1% DMSO. The experiment was prepared in four replicates.

CACO-2 Membrane Permeability Assay.

Measurements were carried out by Absorption Systems LLC. Caco-2 cells (clone C2BBel) were obtained from American Type Culture Collection (Manassas, VA). See details in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Selected examples of known NUDT1 inhibitors and of OGG1 inhibitor SU0268.

Figure 2. Dual NUDT1/OGG1 inhibitor designs. (a) Two design strategies for combining pharmacophores, involving merged structures and linked structures. (b) Initial compound target structures, using merging (**1** and **2**) and linking (**3**) strategies.

Figure 3.

Luminescence probes and assays employed for measurement of NUDT1 and OGG1 activities. (a) ARGO probe for assay of NUDT1 8-oxo-dGTP repair activity and inhibition.

⁵³ (b) OGR1 probe for evaluation of base excision repair activity by OGG1 and inhibition.⁶²

Figure 4.
Dual inhibitor analogs of **3** having reduced hydrophobicity and molecular weight.
Compound **5** is also denoted SU0383.

Figure 5. Docking studies reveal plausible poses of SU0383 in known small-molecule binding pockets of NUDT1 and OGG1. (a) and (b) Modeled structure of murine OGG1 interacting with the tetrahydroquinoline end of SU0383, binding in the same pocket reported for the compound TH5675.³⁸ (c) and (d) Structure of human NUDT1 docked with the aminopyrimidine motif of SU0383.

Figure 6. Titration curves of dual inhibitors in this study. (a) Titration curves for NUDT1 activity of dual inhibitors. (b) Titration curves for OGG1 activity of dual inhibitors. Error bars represent standard deviations from three replicates.

Figure 7. Toxicity testing of **5** (SU0383) by MTT assay with MCF-7 (a) and HeLa (b) cell lines. Data show mean and SD of the viability calculated across five replicates.

Figure 8.

SU0383 (10 μ M) causes increased sensitivity of MCF-7 cells to peroxide (16 mM, 12 h) as measured by cell viability. Data are mean and SD of the viability calculated across four replicates; p value = 0.02 (**), p value = 0.009 (****).

Scheme 1.
Synthesis of Dual NUDT1/OGG1 Inhibitors 3–5

Table 1.IC₅₀ Data of Dual Inhibitors 2–5

compd	IC ₅₀ (μ M)	
	NUDT1	OGG1
2	0.0093	0.62
3	0.0060	2.7
4	0.015	0.94
5	0.034	0.49

Table 2.

CACO-2 Assay with the Dual Inhibitor 5

direction	recovery (%)	P_{app} (10^{-6} cm/s)			efflux ratio
		R1	R2	av	
A-to-B	40	4.78	4.37	4.58	5.1
B-to-A	43	22.6	24.4	23.5	