# Optimization of *N*-piperidinyl-benzimidazolone derivatives as potent and selective inhibitors of 8-Oxo Guanine DNA Glycosylase 1

Olov Wallner,[1, ‡] Armando Cázares-Körner,[1, ‡] Emma Rose Scaletti,[2] Geoffrey Masuyer,[2] Tove Bekkhus,[1] Torkild Visnes,[1, 3] Kirill Mamonov,[1] Florian Ortis,[1] Thomas Lundbäck,[4] Maria Volkova,[1] Tobias Koolmeister,[1] Elisée Wiita,[1] Olga Loseva,[1] Monica Pandey,[1] Evert Homan,[1] Carlos Benítez-Buelga,[1, 5] Jonathan Davies,[2] Martin Scobie,[1, 6] Ulrika Warpman Berglund,[1, 6] Christina Kalderén,[1, 6] Pål Stenmark,[2, 7] Thomas Helleday,[1, 8] Maurice Michel[1, *]

AUTHOR ADDRESS

1 Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

2 Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

3 Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway

4 Chemical Biology Consortium Sweden (CBCS), Science for Life Laboratory, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

5 Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

6 Oxcia AB, Stockholm, Sweden

7 Department of Experimental Medical Science, Lund University, Lund, Sweden

8 Sheffield Cancer Centre, Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

*KEYWORDS: Base excision repair, OGG1, TH5487, cancer, Hit to Lead*

**ABSTRACT:** 8-oxo Guanine DNA Glycosylase 1 is the initiating enzyme within base excision repair and removes oxidized guanines from damaged DNA. Since unrepaired 8-oxoG could lead to G:C→T:A transversion, base removal is of the utmost importance for cells to ensure genomic integrity. For cells with elevated levels of reactive oxygen species this dependency is further increased. In the past we and others have validated OGG1 as a target for inhibitors to treat cancer and inflammation. Here, we present the optimization campaign that led to the broadly used tool compound TH5487. Based on a high-throughput screen, we performed hit to lead expansion and arrived at potent and selective substituted *N*-piperidinyl-benzimidazolones. Using X-ray crystallography data, we describe the surprising binding mode of the most potent member of the class, TH8535. Here, the *N*-Piperidinyl-linker adopts a chair instead of a boat conformation which was found for weaker analogues. We further demonstrate cellular target engagement and efficacy of TH8535 against a number of cancer cell lines.

## Introduction

Of the nucleobases, guanine has the lowest redox potential and the oxidation product, 7,8-dihydro-8-oxoguanine (8-oxoG), has been estimated to be formed in significant quantities (1-10,000 lesions per cell and day).[1] 8-oxoG can base pair with adenine in a *syn* configuration and if the lesion remains unrepaired a G:C→T:A transversion mutation may occur after two replication cycles. This mutagenic cascade can lead to the development of diseases, among them cancer. Evolution has thus equipped humans with an array of enzymes, that prevent 8-oxoG incorporation or foster 8-oxoG excision from DNA.[2] When incorporated into DNA, 8-oxoG is recognized by the enzyme 8-oxo Guanine DNA Glycosylase 1 (OGG1).[2] OGG1 will excise the damaged base and initiate base excision repair.

Considering the higher levels of reactive oxygen species in aberrant cancer cells, they rely on a functional DNA repair system more than normal cells.[3] Consequently, many proteins in the DNA damage response and other pathways are upregulated in cancer and targeting of these proteins has in recent years been an attractive and effective strategy for anticancer drug development.[4,5]

We and others have developed potent OGG1 inhibitors which delay lung inflammation.[6–8] In addition, extensive data has been published on the cellular effects of OGG1 inhibitors in preclinical cancer models.[9–12] Consequently, the tool compound TH5487 has been applied in a number of studies. In addition to genome wide roles, oxidative damage repair at telomeres was shown to be controlled by TH5487.[13] TH5487 also prevents OGG1 recruitment to chromatin[14] and has a potential regulatory function within base excision repair with regard to NEIL1 and NEIL2 as backup enzymes of OGG1.[15]

Here, we outline the medicinal chemistry campaign that led to the discovery of *N*-Piperidinyl-benzimidazolone derivatives as potent inhibitors of OGG1 and describe the screening, hit expansion and structure-activity-relationships (SAR) of the chemical series that led to the discovery of the potent inhibitor TH8535. Further, we provide X-ray crystallographic evidence revealing and rationalizing its binding mode in the active site of OGG1. We then show selectivity against a panel of enzymes involved in base excision repair. We also demonstrate cellular target engagement and efficacy of TH8535 against a panel of cancer cell lines.

## Results

### Identification of *N*-piperidinyl-benzimidazolone as a scaffold for OGG1 Inhibitors

We started screening for OGG1 inhibitors using an in-house developed biochemical assay as reported previously.[6,16] In brief, the assay relies on a fluorophore and a quencher placed opposite one another at the ends of complementary strands of DNA (Figure 1). The more stable OGG1 substrate, 8-oxodA or 8-oxo-adenosine, was placed opposite cytidine, six base pairs distant from the fluorophore. After excision of the nucleobase 8-oxoA by OGG1, a timely incision of the strand was ensured by performing this reaction in the presence of apurinic/apyrimdinic endonuclease 1 (APE1). In the resulting process the fluorophore is separated from proximity to the quencher resulting in a fluorescent signal being generated. Inhibition of OGG1 results in a dose-dependent reduction of that fluorescent signal. Using this set-up, we screened a small molecule library of 17,400 compounds[17] belonging to the Chemical Biology Consortium Sweden as reported before.[6] The screen was carried out using a ligand concentration of 10 µM. In addition to this screening set a library of 600 rule-of-three compliant fragments wase also assayed at 50 µM concentration.

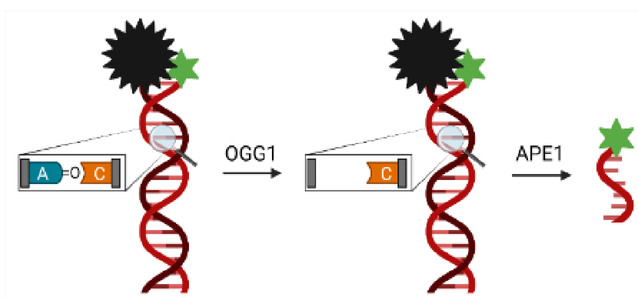



**Figure 1: Principle of the excision-incision coupled assay for OGG1:** 8-oxodA is paired to cytidine in the opposite strand. Six base pairs upstream from 8-oxodA, a fluorophore is placed opposite a quencher in the complementary strand. Excision of the damaged base by OGG1 leads to an abasic site which is incised by APE1. The release of the fluorophore leads to increased fluorescence and is quantified based on reaction progress or inhibition thereof.

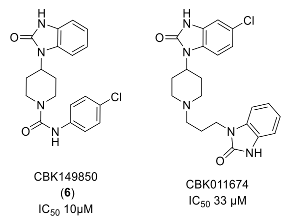
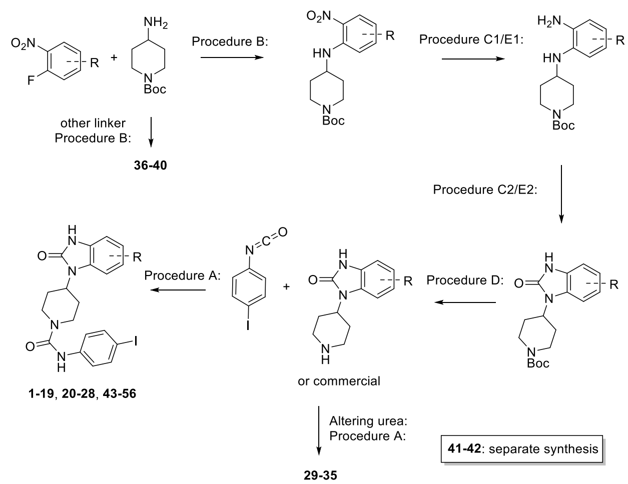



**Figure 2: The two only hits of a HTS campaign to identify OGG1 inhibitors:** CBK149850 has an IC50 of 10 µM and CBK011674, also known as Domperidone, is slightly less potent with an IC50 of 33 µM.

Visual assessment of the hits was performed, excluding frequent hitters, such as redox cyclers and chelating substances. A dose-response characterization was performed for compounds of interest that reached 40% enzyme inhibition at 10 µM. The remaining compounds were then counter-screened for APE1 inhibition and DNA intercalation and tested for identity and purity by LCMS.[16] As a result, two structurally related compounds were identified as the only hits. CBK149850 with an IC50 of 10 µM and CBK011674, also known as Domperidone, with an IC50 of 33 µM (Figure 2). Interestingly, both structures were based on a piperidinylbenzimidazolone motif. Considering the potency of CBK149850, it was decided to initially explore the SAR by preparing a series of aniline-based ureas for hit-to-lead expansion. The scope of this investigation was further broadened by utilising the 5-chloro substituted benzimidazolone embodied in Domperidone.

## Iterative chemistry and biochemical evaluation

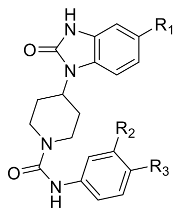
Both, 5-chloro-1-(4-piperidinyl)-2-benzimidazolone and 4-(2-keto-1-benzimidazolinyl)-piperidine are commercially available reagents and were used to generated matched pairs using the corresponding isocyanates and DIPEA in DCM (Procedure A, Scheme 1).




**Scheme 1: Synthetic route to substituted *N*-Piperidinyl-benzimidazolone derivatives:** Altering the aniline part of the molecule was modular and readily achieved over a single step (1-19 and 43-56). Diversification within the benzimidazolone core or the linker required individual synthesis over five steps (20-28, 29-35 and 36-40).

Table 1 summarizes thermal stabilization of OGG1 using compounds **1-19** as measured by differential scanning flourimetry (DSF)[18] together with the activity of these compounds in the biochemical assay (IC50). Analogues incorporating the unsubstituted benzimidazolone scaffold were comparably less potent than their 5-chloro-substituted counter-parts, among them resynthesized CBK149850, compound **6**. In addition, a clear preference was observed for 4-halogen-substituted aniline derivatives. This tendency was also observed for both scaffolds in the DSF assay, indicating a strong interaction with the target at this position. Since, 4-iodo and 3,4-dichloro substitution in **8** and **9** as well as **18** and **19** yielded the highest potency, we decided to next combine this modification with an investigation into the tolerated groups within the benzimidazolone scaffold.

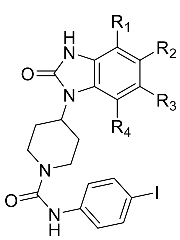
**Table 1: Summary of alteration of the aniline part of the urea moiety; stabilization in DSF (ΔT) in K and biochemical inhibition ($IC_{50}$ with confidence intervals 95) in µM. * single replicate**



| # | $R_1$ | $R_2$ | $R_3$ | DSF | $IC_{50}$ ($CI_{95}$) |
|---|---|---|---|---|---|
| **1** | H | H | H | 0.4 | >100 |
| **2** | H | F | H | 1.6 | >100 |
| **3** | H | Cl | H | 1.2 | >100 |
| **4** | H | H | Me | 3.1 | 19.9 (11.6 - 34.3) |
| **5** | H | H | F | 1.3 | >100 |
| **6** | H | H | Cl | 4.6 | 5.5 (4.1 - 7.2) |
| **7** | H | H | Br | 2.1 | 7.0 (1.8 - 27.8) |
| **8** | H | H | I | 2.4 | 2.9 (2.3 - 3.7) |
| **9** | H | Cl | Cl | 4.7 | 2.9 (1.5 - 5.5) |
| **10** | H | H | $NO_2$ | 0.2 | >100 |
| **11** | Cl | H | H | 3.3 | 41.9 (38.3 - 45.7) |
| **12** | Cl | F | H | 2.4 | 35.1 (34.6 - 35.6) |
| **13** | Cl | Cl | H | 1.3 | >100 |
| **14** | Cl | H | Me | 1.5 | >100 |
| **15** | Cl | H | F | 1.7 | no effect |
| **16** | Cl | H | Cl | 3.8 | 2.0 (1.2 - 3.4) |
| **17** | Cl | H | Br | 4.1 | 1.6* |
| **18** | Cl | H | I | 1.6 | 1.4 (0.78 - 2.6) |
| **19** | Cl | Cl | Cl | 3.1 | 1.3 (1.3 - 1.4) |

We set out to probe the functional group tolerance of a single methyl group around the indicated positions $R_1$ to $R_4$ and synthesized the required methyl substituted intermediate materials according to Scheme 1. Starting from the corresponding fluoro-nitro-toluene we performed aromatic substitution with Boc-protected 4-amino-piperidine using DIPEA in isopropanol. Next, the nitro group was reduced on Pd/C with hydrogen in THF and the resulting diamine was cyclized using Triphosgene and DIPEA. As a last step, the protecting group was removed using TFA. The intermediate *N*-piperidinyl-benzimidazolones were coupled with 4-iodo-phenyl-isocyanate as above giving compounds **20-23**.

**Table 2: Investigation of tolerated substitutions at the benzimidazolone core; stabilization in DSF (ΔT) in K and biochemical inhibition ($IC_{50}$ with confidence interval 95) in µM. * single replicate**



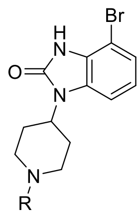
| # | $R_1$ | $R_2$ | $R_3$ | $R_4$ | DSF | $IC_{50}$ ($CI_{95}$) |
|---|---|---|---|---|---|---|
| **20** | Me | H | H | H | 3.8 | 0.63 (0.55 - 0.72) |
| **21** | H | Me | H | H | 3.8 | 1.7 (1.4 - 2.0) |
| **22** | H | H | Me | H | 3.4 | no effect |
| **23** | H | H | H | Me | 2.2 | 8.7* |
| **24** | F | H | H | H | 3.9 | 2.2 (1.7 - 2.7) |
| **25** | Cl | H | H | H | 2.0 | 2.7 (2.0 - 37.2) |
| **TH5487 (26)** | Br | H | H | H | 4.1 | 0.30 (0.24 - 0.40) |
| **27** | OMe | H | H | H | 4.2 | 0.91 (0.57 - 1.4) |
| **TH5675 (28)** | $NH_2$ | H | H | H | 5.5 | 0.75 (0.33 - 1.7) |

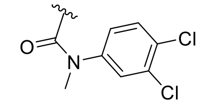
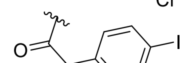
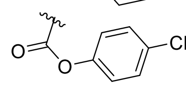
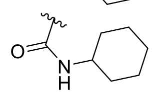
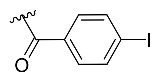
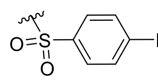
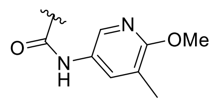
Investigation in the biochemical assay revealed **20** to stand out with a potency of 630 nM (Table 2). The potency dropped to 1.7 µM (**21**), inactivity (**22**) and 8.7 µM (**23**) for the other positions, indicating that $R_1$ modification provided the most tolerated substitution within the inhibitor. Based on these findings we assessed alternative substituents replacing the methyl group, including halides, as well as a methoxy and an amine group. The synthetic efforts yielded five more analogues **24-28** (Table 2). The biochemical assay confirmed 4-position modified analogues as potent OGG1 inhibitors. Among these products were bromide **26** and amine **28** with potencies of 300 nM and 750 nM. The increased thermal shift for these analogues in DSF further indicated a preferential interaction with the target protein.

With the 4-iodoaniline at the one end and 4-Br-substitution within the benzimidazolone at the other end set, we directed our attention towards the middle part of the molecule. Synthetically, 4-bromo-1-(4-piperidinyl)-2-benzimidiazolone derivatives were readily accessible with the established chemistry. Consequently, we first assessed, whether the urea moiety was required for potency. For that, we generated other functional groups as urea alternatives by broadening the synthetic scope of the strategy in Scheme 1.

Among the products **29-35** were amides, carbamates, sulfonamides as well as other modified ureas (Table 3).

**Table 3: Results of investigating urea alternatives; stabilization in DSF (ΔT) in K and biochemical inhibition ($IC_{50}$ with confidence interval 95) in μM. * single replicate**

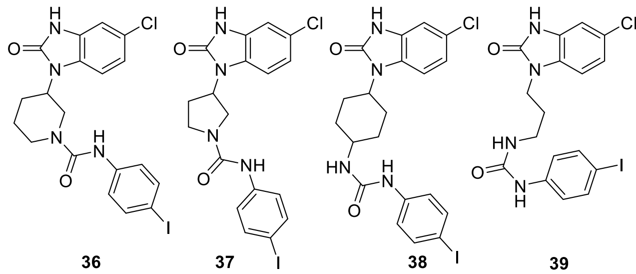



| # | R | DSF | $IC_{50}$ (CI 95) |
|---|---|---|---|
| **29** |  | 0.0 | no effect |
| **30** |  | -0.73 | no effect |
| **31** |  | 0.1 | no effect |
| **32** |  | 0.43 | >100 |
| **33** |  | -0.1 | no effect |
| **34** |  | -0.5 | 14* |
| **35** |  | 4.5 | 4.4* |

Here, the analogues provided revealed that N-methylation in **29**, a methylene group in **30**, the carbamate in **31**, as well as 4-iodo-benzoic- **33** and –benzo-sulfonic acid **34** modification resulted in no or a very low activity against OGG1. This suggested that the particular nitrogen of the formerly used aniline derivatives undergoes an important beneficial interaction with OGG1. In addition, a cyclohexylamine **32** and a 3-aminopyridyl derivative **35** were only weakly active confirming the need for a non-hetero atom containing $sp^2$-system attached to a urea moiety.

Next we focused our attention towards the *N*-piperidinyl linker. Following the chemistry from above, we generated 4-iodo-aniline ureas with different linkers towards the 4-bromo-benzimidazolone. None of the produced analogues **36-40** reached nM potency and no stabilization was observed in DSF (Table 4). In addition, we altered the benzimidazolone moiety by generating the corresponding benzimidazole **41**, as well as a benzoxazolone variant **42**, neither of which had a pronounced effect on inhibiting or stabilizing OGG1 (Table 4).

**Table 4: Alternatives for *N*-piperidinyl-linker and benzimidazolone core are summarized; stabilization in DSF (ΔT) in K and biochemical inhibition ($IC_{50}$ with confidence intervals 95) in µM. * not applicable**

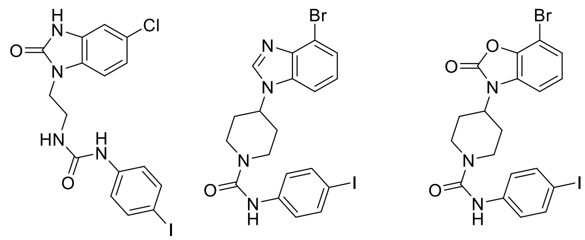


**36** no effect, DSF -0.27 K

**37** no effect, DSF -0.13 K

**38** no effect, DSF -0.81 K

**39** 12.7 (8.9 - 18.2), DSF 0.0



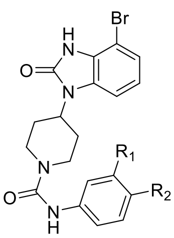
**40** 20.6 (16.3 - 25.9), DSF 0.0

**41** >100, DSF *

**42** >100, DSF 0.50 K

With that, all optimization attempts of the linker including replacement of the urea function, modification of the *N*-piperidinyl spacer as well as alterations to the benzimidazolone core were unsuccessful. Thus, we revisited the tolerated aniline derivatives within the urea moiety using chemistry from above and generated analogues **43-56** (Table 5).

**Table 5: Summary of revisiting tolerated aniline derivatives within the urea moiety; stabilization in DSF (ΔT) in K and biochemical inhibition ($IC_{50}$ with confidence interval 95) in µM. * single replicate, - no applicable**



| # | $R_1$ | $R_2$ | DSF | $IC_{50}$ ($CI_{95}$) |
|---|---|---|---|---|
| **43** | H | Cl | 4.2 | 0.35 (0.24 -0.50) |
| **44** | H | Br | 4.2 | 0.19 (0.14 - 0.28) |
| **45** | H | Me | 5.1 | 2.1 (1.7 - 2.7) |
| **46** | H | *t*-Bu | 0.0 | 46 ($Y_{max}$ 57%) |
| **47** | H | OMe | 4.1 | 3.1* |
| **48** | H | $NO_2$ | -0.17 | no effect |
| **49** | H | $CF_3$ | 1.6 | 1.6 (1.4 - 1.7) |
| **50** | H | COOEt | -0.11 | no effect |
| **51** | H | NHAc | -0.37 | no effect |
| **52** | H | $NMe_2$ | - | 32.2 (30.3 - 34.1) |
| **53** | Cl | Cl | 3.1 | 0.44 (0.30 - 0.66) |
| **54** | OMe | Cl | 1.9 | 0.30 (0.17 - 0.52) |
| **TH8535 (55)** | OMe | Me | 1.8 | 0.20 (0.11 - 0.36) |
| **56** | OMe | OMe | 5.1 | 4.4 (3.5 - 5.5) |

Again, the 4-halogen substituents (**43**, **44**, **53** and **54**) were clear stand outs, with all of them having nM potencies between 440 nM and 190 nM. Compound **55** (TH8535) incorporates 4-methylation with an additional 3-methoxy substitution and reaches 200 nM potency. No other substituents within the aniline moiety conveyed any noticeable activity. Neither bulky alkyl substitution nor hydrogen bond donors or acceptor resulted in potent analogs (**46**, **48 – 52**).

## TH8535 is a selective OGG1 inhibitor

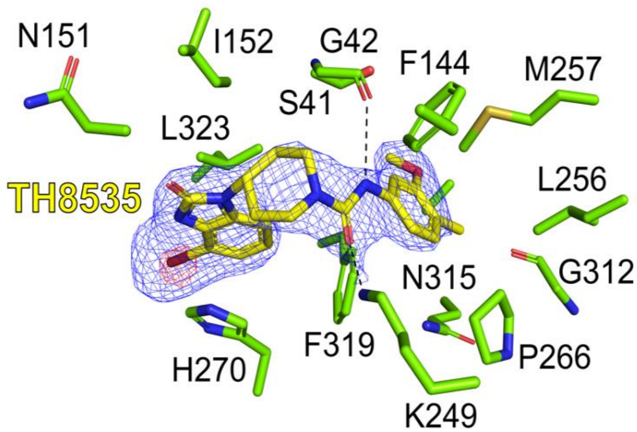



**Figure 3. The recognition of TH8535 by OGG1.** Amino acids contributing to ligand binding are depicted as sticks; C atoms are colored green, O atoms red, N atoms blue and S atoms gold. TH8535 is presented as a stick model; C atoms colored yellow and Br atoms colored burgundy. Hydrogen bond interactions are shown as dashed lines. The $2F_o$-$F_c$ electron density map around TH8535 is contoured at 1.0 σ (blue) and the $F_o$-$F_c$ electron density maps are contoured at -3.0 σ (red) and +3.0 σ (green).

With TH8535 identified as a potent OGG1 inhibitor, we assessed the compound and 4-halogen substituted members of the series for their activity against a number of DNA glycosylases and base excision repair enzymes (Table 6). Of the tested enzymes, only SMUG1 was weakly inhibited by all tested compounds ($IC_{50}$>100 µM). In addition, three compounds showed weak inhibition of Fpg with $IC_{50}$ above 100 µM, confirming the series to be selective inhibitors within the base excision repair pathway.

**Table 6: Selectivity of potent members of the inhibitor series against a panel of DNA glycosylases and APE1. IC50 in µM;** No effect – n.e.

| | APE1 | NEIL1 | Fpg | UNG2 | TDG | SMUG1 |
|---|---|---|---|---|---|---|
| **26, TH5487** | n. e. | n. e. | >100 µM | n. e. | n. e. | >100 µM |
| **43** | n. e. | n. e. | >100 µM | n. e. | n. e. | >100 µM |
| **44** | n. e. | n. e. | >100 µM | n. e. | n. e. | >100 µM |
| **53** | n. e. | n. e. | n. e. | n. e. | n. e. | >100 µM |
| **54** | n. e. | n. e. | n. e. | n. e. | n. e. | >100 µM |
| **55, TH8535** | n. e. | n. e. | n. e. | n. e. | n. e. | >100 µM |

## Co-crystal structure of TH8535 and OGG1

To investigate the binding mode, we obtained the crystal structure of TH8535 in complex with OGG1 showing active site binding at 2.45 Å resolution. The urea moiety of the inhibitor is positioned between two hydrogen bonds involving the backbone oxygen of G42 and the sidechain of K249 (Figure 3). In addition, TH8535 is supported by extensive hydrophobic interactions with S41, F144, N151, I152, L256, M257, P266, H270, G312, N315, F319 and L323. The residues H270 and F319 aid in the positioning of TH8535 through important CH/π and π-stacking interactions. In addition, we obtained the crystal structure of TH9525, a less potent analogue, which bound in a similar orientation as TH8535 (Figure 4). The difference in molecular structure between TH9525 and TH8535 is a methoxy group instead of a bromide at the benzimidazolone with no obvious interaction with protein residues beyond hydrophobic contact. However subtle, this difference led to a thirteen fold drop in $IC_{50}$ from 200 nM (TH8535) to 2.0 µM (TH9525).

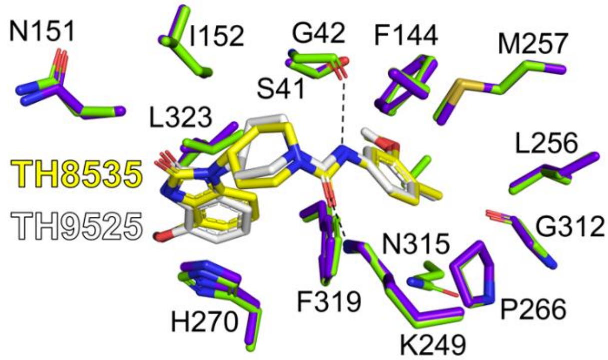



**Figure 4: The active site hydrogen bond network of TH8535 bound OGG1 compared to TH9525.** Amino acids contributing to ligand binding are depicted as sticks; C atoms are colored green (OGG1-TH8535) or purple (OGG1-TH9525), O atoms red, N atoms blue and S atoms gold. TH8535 and TH9525 are presented as stick models; C atoms colored yellow (OGG1-TH8535) or white (OGG1-TH9525) and Br atoms colored burgundy. Hydrogen bond interactions between OGG1 and TH9525 are shown as dashed lines.

## Potent OGG1 inhibitors adopt a chair conformation

This observation prompted us to computationally investigate the protein residue network and its dynamics upon binding of weak and strong inhibitors. We prepared the mouse OGG1 protein complexes with TH8535, TH9525 and TH5675 as well as human OGG1 protein with TH5487 with Maestro and inspected the resulting structures for differences. As the least potent member, TH9525 was the only structure adopting a twisted boat instead of a chair conformation within the *N*-piperidinyl-linker (Figure 5).[6,9] The visible result is a movement of the benzimidazolone core towards the flank of the binding pocket, establishing an aromatic H-bond with D322 while simultaneously losing the H-bond with I152. Interestingly, when investigating the resulting structural changes using molecular dynamics calculations, we observed that the change in conformation appears to also disrupt an amino acid network of H270, D268 and K249 by altering intra-protein-interactions. As a result, the K249-urea-H-bond is destabilised and thus TH9525 exhibits lower affinity to OGG1 than its chair adopting analogues TH8535, TH5675 and TH5487 (Supporting Figure 1).

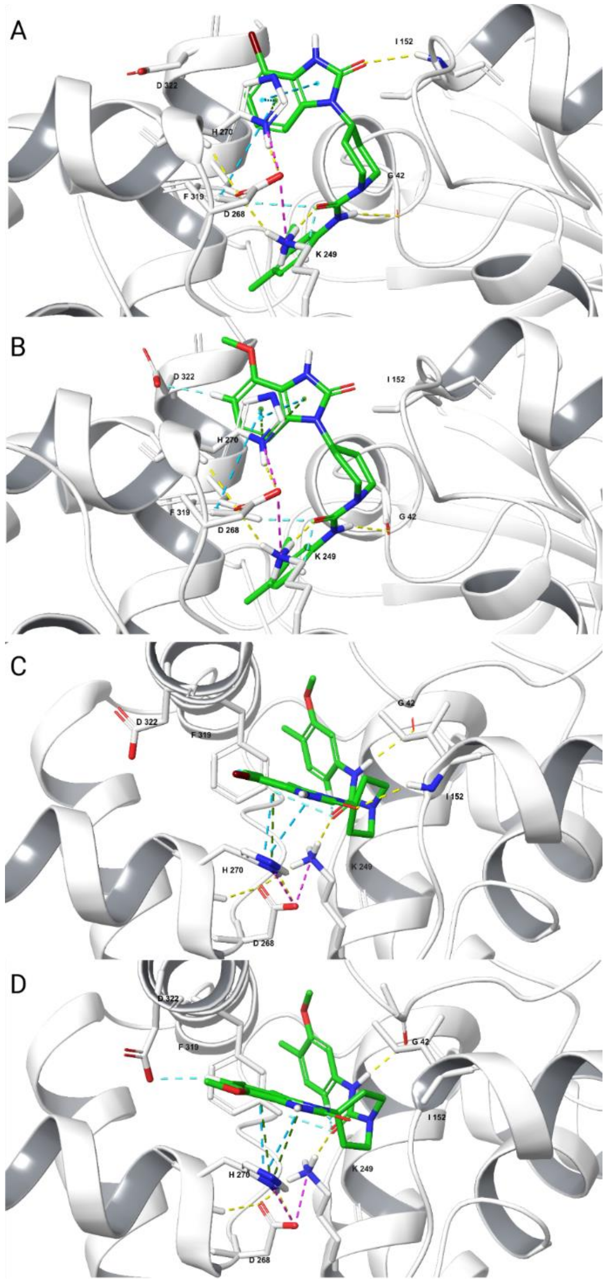



**Figure 5: *N*-Piperidinyl-based inhibitors OGG1 adopt a chair (TH8535, potent) or a boat conformation (TH9525, weak). A**) Side view of TH8535. The compound is in chair conformation and builds a beneficial H-bond with the amide of I152; **B**) Side view of TH9525. A twisted boat conformation is adopted by the compound pushing it to the flank of the pocket, altering interactions with active site amino acids I152, D322, H270, D68 and K249; **C**) Top view of TH8535; **D**) Top view of TH9525.

In addition and due to the size of the heteroatom, 4-iodo-aniline substitution at the urea results in less deep binding within the active site. This partly counteracts the benefit gained from a chair conformation, as evident in the crystal structures of TH5675 and TH5487 were the latter forms a halogen bond with Gly312 but also has fewer interactions within the axis H270-D268-K249 (Supporting Information).

## TH8535 is potent against a panel of cancer cells

We next assessed the intracellular efficacy ($EC_{50}$) of TH8535. Consequently, cellular target engagement was confirmed using CETSA, showing a strong stabilization of OGG1 by both TH5487 and TH8535 in HL60 cells (Supplementary Information).[19] We then determined the sensitivity of a panel of cancer and non-transformed cell lines towards TH8535. We found that TH8535 caused a significant loss of viability in a number of cancer cells while being better tolerated in non-transformed cell lines (Figure 6).

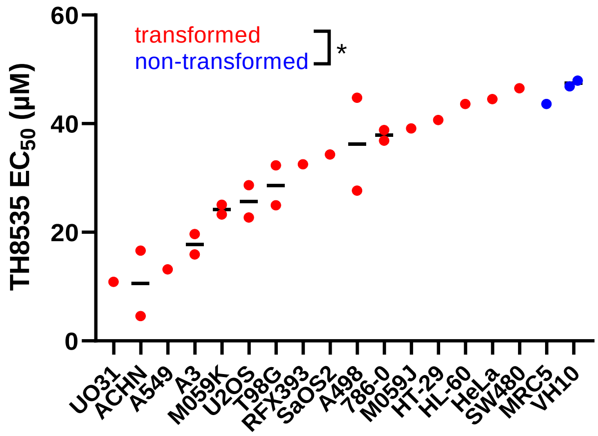



**Figure 6: Viability of transformed and non-transformed cell lines after being exposed to TH8535:** EC50 values obtained in 16 cancer (red) and 2 non-transformed cell lines (blue). Cells were exposed to a dilution series of TH8535 for five days followed by a viability assessment using resazurin. Each point represents the EC50-value from one experiment (average of two or three technical replicates); *, $p < 0.05$.

## Discussion and Conclusions

Here we reported the HTS and hit-to-lead expansion on the discovery of inhibitors of OGG1. We identified substituted *N*-Piperidinyl-benzimidalones as weak inhibitors and optimized the series to its most potent member TH8535. With an IC50 of 200 nM the compound is selective over other enzymes within base excision repair, engages OGG1 in cells and selectively decreases the viability of transformed cell lines.

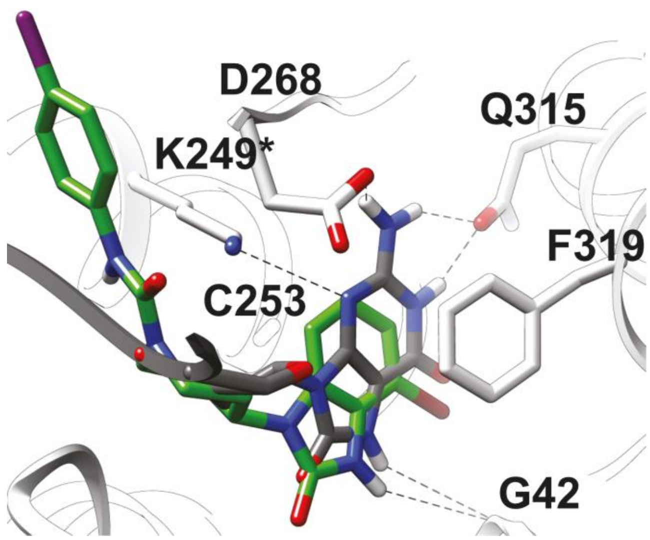



**Figure 7: Overlay of TH5487 and 8-oxoG containing DNA with OGG1 as based on molecular docking (TH5487) or crystal structure (OGG1 and DNA substrate, PDB: 1EBM).** Amino acids highlighted contribute to protein-ligand/substrate interactions. The binding mode predicted by computational docking overlays the benzimidazolone core with the flipped out 8-oxoG. The protein is shown in white, TH5487 in green, substrate in grey, O in red, N in blue, I in purple, * catalytic lysine 249.

During the SAR investigation no structural proof of ligand-protein interaction was obtained. In the absence of such data, we investigated ligand binding using hydrogen-deuterium-exchange mass spectrometry (HDX-MS). Here, we demonstrated engagement of OGG1 peptides close to the active site by TH5487 as reported earlier.[6] In addition we performed molecular docking of the ligand into an available OGG1 crystal structure (PDB: 1EBM, Figure 7). In this simulation, TH5487 mimicked the 8-oxo-guanosine substrate with the benzimidazoline core and engaged the outer part of the binding pocket with the 4-iodoaniline substituent.

Only after arriving at TH8535 and broadly studying tool compound TH5487 we succeeded in resolving the co-crystal structures of OGG1 in complex with TH5487 or TH5675.[6,9] As reported by Visnes *et al.* in 2018 and Visnes *et al.* in 2020, TH5675 and TH5487 are OGG1 active site inhibitors. To our surprise, all of the OGG1 inhibitor co-crystal structures showed an inversed binding orientation of the crystallized inhibitors compared to the results of their respective *in silico* study. While modelling pointed towards an overlapping orientation of the benzimidazolone with the position of the canonical 8-oxoG base, the co-crystal structure revealed the piperidinyl-urea linker interacts with thus part of the enzyme instead.

Using molecular dynamics simulations to further guide ligand optimization, we assessed the relevance of singular protein-ligand interactions by residence time of singular protein-ligand contacts. This demonstrated the relevance of amino acid interactions that were established while expanding the chemical series (Supporting Figure 1). Here, 4-iodo-aniline based inhibitors undergo a halogen bond with G312 that was reflected in early DSF and biochemical data (Table 1). Within the urea linker, it is G42 and K249 that establish H-bonds with the urea (Table 3, Figure 3 and 4). F319 addresses both the aniline ring and the benzimidazolone via π-stacking, with the latter also coordinated by H270. These amino acids involved in binding the inhibitor series are identical with those relevant for the enzymatic reaction performed by OGG1.[20] Interestingly, while investigating the complexes before simulation we observed both boat and chair conformations within the *N*-piperidinyl-linker among the inhibitors co-crystallized with OGG1. A chair conformation was preferred by more potent inhibitors, among them TH8535, TH5675 and TH5487. Adopting the boat conformation in turn distorted protein-ligand interactions with H270 and surprisingly also with K249 through affecting a tight amino acid network, leading to weaker inhibitors. This effect was also partially observable for 4-iodoaniline modified ureas. The possibility of forming a halogen bond between iodine and G312 led to a less deep binding, mimicking the amino acid network distortion of a boat conformation. This points towards the use of 3-methoxy-4-methyl-anilines within the urea and a chair adopting *N*-piperidinyl-linker as preferable modifications to optimize the ligand-OGG1 interactions.

Collectively, the SAR, structural proof and molecular simulations presented here, demonstrate that potent OGG1 inhibitors address amino acids that are involved in DNA strand stabilization, substrate orientation and base excision mechanisms of OGG1.

## AUTHOR INFORMATION

### Corresponding Author

* Maurice Michel: maurice.michel@ki.se
Science for Life Laboratory, Department of Oncology and Pathology, Karolinska Institutet

### Author Contributions

O.W., A.C.K., K.M., F.O., T.L., M.V., T.K., M.S. and M.M. have planned, contributed and analyzed medicinal chemistry experiments. T.V., A.C.K., T.L., E.W., O.L., M.M. have planned, contributed and analyzed biochemical experiments. E.R.S., G.M., J.S., P.S. have planned, contributed and analyzed structural biology experiments. A.C.K., E.H. and M.M. have planned, contributed and analyzed computational chemistry experiments. T.B., T.V., C.B.B., M.P., U.W.B., C.K. and T.H. have planned, contributed and analyzed biological experiments. The manuscript was written by O.W. and M.M. All authors have given approval to the final version of the manuscript. / ‡These authors contributed equally.

### Funding Sources

This work was funded by The European Research Council (TAROX Programme), The Swedish Research Council (2015-00162 T.H., 2018-03406 P.S.), The Alfred Österlund foundation (P.S.), The Crafoord foundation (20190532 P.S.), The Swedish Pain Relief Foundation, The Swedish Cancer Society (CAN2018/0658 T.H., CAN 2017/716 P.S.), the Torsten and Ragnar Söderberg Foundation (T.H.), the Åke-Olsson foundation for haematological research (2020-00306 M.M.), Postdoctoral Stipends of the Thomas Helleday Foundation for medical research (M.M. and C.B.B.) and Karolinska Institutets Research Foundation Grant (2020-02186 M.M.). This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 875510 (M.M., E.J.H. E.W.) The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and Ontario Institute for Cancer Research, Royal Institution for the Advancement of Learning McGill University, Kungliga Tekniska Hoegskolan, Diamond Light Source Limited. This communication reflects the views of the authors and the JU is not liable for any use that may be made of the information contained herein

**Notes**

T.V., A.C.K., O.W., T.K., and T.H. are listed as inventors on a U.S. patent no. WO2019166639A1, covering OGG1 inhibitors. The patent is fully owned by a non-profit public foundation, the Helleday Foundation, and T.H. and U.W.B. are members of the foundation board. U.W.B., C.K. and M.S. are employees of Oxcia, a company developing OGG1 inhibitor towards the clinics. T.L. is an employee of Astra Zeneca and has performed experiments for this manuscript while working for Chemical Biology Consortium Sweden. The remaining authors declare no competing financial interests.

## ACKNOWLEDGMENTS

Celestine N. Chi at Uppsala University is acknowledged for NMR services. We thank the Chemical Biology Consortium Sweden for support with small molecule libraries, screening and assay set-up. We would like to thank the protein science facility at Karolinska Institutet and Mark Thompson at the University of Sheffield for preparing enzyme stocks. Mari Kullman Magnusson, Louise Sjöholm, Therese Pham, Sofie Demir, Teresa Sandvall, Kristina Edfeldt, Dana Michel, Franz Ferdinand and Michael Sundström are acknowledged for technical and administrative support and critical discussions. We thank MAXIV Laboratory (Sweden, proposal MX20200204), Diamond Light Source (United Kingdom, proposals MX15806 and MX21625), and their beamline scientists from the BioMAX, I04 and I04-1 beamlines for their support during data collection.

## ABBREVIATIONS

OGG1, 8-oxo-Guanine DNA Glycosylase 1; APE1, apurininc/apyrimdinic Endonuclease 1; 8-oxoG, 8-oxo-guanine; 8-oxodA, 8-oxo-adenosine; 8-oxoA, 8-oxo-adenine; NEIL1/2, Endonuclease VIII-like ½; SAR, Structure-Activity Relationship; DSF, Differential Scanning Fluorimetry; Fpg, Formamidopyrimidine DNA Glycosylase; UNG2, Uracil DNA Glycosylase; TDG, G/T mismatch-specific Thymine DNA Glycosylase; SMUG1, Single-Strand-Selective Monofunctional Uracil DNA glycosylase

## REFERENCES

(1) Nakabeppu, Y. Cellular Levels of 8-Oxoguanine in Either DNA or the Nucleotide Pool Play Pivotal Roles in Carcinogenesis and Survival of Cancer Cells. *International Journal of Molecular Sciences* **2014**, *15* (7), 12543–12557. https://doi.org/10.3390/ijms150712543.

(2) Visnes, T.; Grube, M.; Hanna, B. M. F.; Benitez-Buelga, C.; Cázares-Körner, A.; Helleday, T. Targeting BER Enzymes in Cancer Therapy. *DNA Repair (Amst)* **2018**, *71*, 118–126. https://doi.org/10.1016/j.dnarep.2018.08.015.

(3) Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144* (5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013.

(4) O'Connor, M. J. Targeting the DNA Damage Response in Cancer. *Molecular Cell* **2015**, *60* (4), 547–560. https://doi.org/10.1016/j.molcel.2015.10.040.

(5) Blackford, A. N.; Jackson, S. P. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Molecular Cell* **2017**, *66* (6), 801–817. https://doi.org/10.1016/j.molcel.2017.05.015.

(6) Visnes, T.; Cázares-Körner, A.; Hao, W.; Wallner, O.; Masuyer, G.; Loseva, O.; Mortusewicz, O.; Wiita, E.; Sarno, A.; Manoilov, A.; Astorga-Wells, J.; Jemth, A.-S.; Pan, L.; Sanjiv, K.; Karsten, S.; Gokturk, C.; Grube, M.; Homan, E. J.; Hanna, B. M. F.; Paulin, C. B. J.; Pham, T.; Rasti, A.; Berglund, U. W.; Nicolai, C. von; Benitez-Buelga, C.; Koolmeister, T.; Ivanic, D.; Iliev, P.; Scobie, M.; Krokan, H. E.; Baranczewski, P.; Artursson, P.; Altun, M.; Jensen, A. J.; Kalderén, C.; Ba, X.; Zubarev, R. A.; Stenmark, P.; Boldogh, I.; Helleday, T. Small-Molecule Inhibitor of OGG1 Suppresses Proinflammatory Gene Expression and Inflammation. *Science* **2018**, *362* (6416), 834–839. https://doi.org/10.1126/science.aar8048.

(7) Qin, S.; Lin, P.; Wu, Q.; Pu, Q.; Zhou, C.; Wang, B.; Gao, P.; Wang, Z.; Gao, A.; Overby, M.; Yang, J.; Jiang, J.; Wilson, D. L.; Tahara, Y.; Kool, E. T.; Xia, Z.; Wu, M. Small-Molecule Inhibitor of 8-Oxoguanine DNA Glycosylase 1 Regulates Inflammatory Responses during Pseudomonas Aeruginosa Infection. *The Journal of Immunology* **2020**. https://doi.org/10.4049/jimmunol.1901533.

(8) Tanner, L.; Single, A. B.; Bonghir, R. K. V.; Oomen, R.; Wallner, O.; Helleday, T.; Kalderen, C.; Egesten, A. *Small-Molecule-Mediated OGG1 Inhibition Attenuates Pulmonary Inflammation and Lung Fibrosis*; 2021; p 2021.02.27.433075. https://doi.org/10.1101/2021.02.27.433075.

(9) Visnes, T.; Benítez-Buelga, C.; Cázares-Körner, A.; Sanjiv, K.; Hanna, B. M. F.; Mortusewicz, O.; Rajagopal, V.; Albers, J. J.; Hagey, D. W.; Bekkhus, T.; Eshtad, S.; Baquero, J. M.; Masuyer, G.; Wallner, O.; Müller, S.; Pham, T.; Göktürk, C.; Rasti, A.; Suman, S.; Torres-Ruiz, R.; Sarno, A.; Wiita, E.; Homan, E. J.; Karsten, S.; Marimuthu, K.; Michel, M.; Koolmeister, T.; Scobie, M.; Loseva, O.; Almlöf, I.; Unterlass, J. E.; Pettke, A.; Boström, J.; Pandey, M.; Gad, H.; Herr, P.; Jemth, A.-S.; El Andaloussi, S.; Kalderén, C.; Rodriguez-Perales, S.; Benítez, J.; Krokan, H. E.; Altun, M.; Stenmark, P.; Berglund, U. W.; Helleday, T. Targeting OGG1 Arrests Cancer Cell Proliferation by

Inducing Replication Stress. *Nucleic Acids Research* **2020**, *48* (21), 12234–12251. https://doi.org/10.1093/nar/gkaa1048.
(10) Tahara, Y.; Auld, D.; Ji, D.; Beharry, A. A.; Kietrys, A. M.; Wilson, D. L.; Jimenez, M.; King, D.; Nguyen, Z.; Kool, E. T. Potent and Selective Inhibitors of 8-Oxoguanine DNA Glycosylase. *J. Am. Chem. Soc.* **2018**, *140* (6), 2105–2114. https://doi.org/10.1021/jacs.7b09316.
(11) Donley, N.; Jaruga, P.; Coskun, E.; Dizdaroglu, M.; McCullough, A. K.; Lloyd, R. S. Small Molecule Inhibitors of 8-Oxoguanine DNA Glycosylase-1 (OGG1). *ACS Chem. Biol.* **2015**, *10* (10), 2334–2343. https://doi.org/10.1021/acschembio.5b00452.
(12) Rietdijk, J.; Tampere, M.; Pettke, A.; Georgiev, P.; Lapins, M.; Warpman-Berglund, U.; Spjuth, O.; Puumalainen, M.-R.; Carreras-Puigvert, J. A Phenomics Approach for Antiviral Drug Discovery. *BMC Biology* **2021**, *19* (1), 156. https://doi.org/10.1186/s12915-021-01086-1.
(13) Baquero, J. M.; Benítez-Buelga, C.; Rajagopal, V.; Zhenjun, Z.; Torres-Ruiz, R.; Müller, S.; Hanna, B. M. F.; Loseva, O.; Wallner, O.; Michel, M.; Rodríguez-Perales, S.; Gad, H.; Visnes, T.; Helleday, T.; Benítez, J.; Osorio, A. Small Molecule Inhibitor of OGG1 Blocks Oxidative DNA Damage Repair at Telomeres and Potentiates Methotrexate Anticancer Effects. *Scientific Reports* **2021**, *11* (1), 3490. https://doi.org/10.1038/s41598-021-82917-7.
(14) Hanna, B. M. F.; Helleday, T.; Mortusewicz, O. OGG1 Inhibitor TH5487 Alters OGG1 Chromatin Dynamics and Prevents Incisions. *Biomolecules* **2020**, *10* (11), 1483. https://doi.org/10.3390/biom10111483.
(15) Hanna, B. M. F.; Michel, M.; Helleday, T.; Mortusewicz, O. NEIL1 and NEIL2 Are Recruited as Potential Backup for OGG1 upon OGG1 Depletion or Inhibition by TH5487. *International Journal of Molecular Sciences* **2021**, *22* (9), 4542. https://doi.org/10.3390/ijms22094542.
(16) Michel, M.; Visnes, T.; Homan, E. J.; Seashore-Ludlow, B.; Hedenström, M.; Wiita, E.; Vallin, K.; Paulin, C. B. J.; Zhang, J.; Wallner, O.; Scobie, M.; Schmidt, A.; Jenmalm-Jensen, A.; Warpman Berglund, U.; Helleday, T. Computational and Experimental Druggability Assessment of Human DNA Glycosylases. *ACS Omega* **2019**, *4* (7), 11642–11656. https://doi.org/10.1021/acsomega.9b00162.
(17) Michel, M.; Homan, E. J.; Wiita, E.; Pedersen, K.; Almlöf, I.; Gustavsson, A.-L.; Lundbäck, T.; Helleday, T.; Warpman Berglund, U. In Silico Druggability Assessment of the NUDIX Hydrolase Protein Family as a Workflow for Target Prioritization. *Front. Chem.* **2020**, *8*. https://doi.org/10.3389/fchem.2020.00443.
(18) Niesen, F. H.; Berglund, H.; Vedadi, M. The Use of Differential Scanning Fluorimetry to Detect Ligand Interactions That Promote Protein Stability. *Nature Protocols* **2007**, *2* (9), 2212–2221. https://doi.org/10.1038/nprot.2007.321.
(19) Jafari, R.; Almqvist, H.; Axelsson, H.; Ignatushchenko, M.; Lundbäck, T.; Nordlund, P.; Martinez Molina, D. The Cellular Thermal Shift Assay for Evaluating Drug Target Interactions in Cells. *Nat Protoc* **2014**, *9* (9), 2100–2122. https://doi.org/10.1038/nprot.2014.138.
(20) Fromme, J. C.; Bruner, S. D.; Yang, W.; Karplus, M.; Verdine, G. L. Product-Assisted Catalysis in Base-Excision DNA Repair. *Nat Struct Mol Biol* **2003**, *10* (3), 204–211. https://doi.org/10.1038/nsb902.

Supporting Information

# Optimization of *N*-piperidinyl-benzimidazolone derivatives as potent and selective inhibitors of 8-Oxo Guanine DNA Glycosylase 1

Olov Wallner,[1,‡] Armando Cázares-Körner,[1,‡] Emma Rose Scaletti,[2] Geoffrey Masuyer,[2] Tove Bekkhus,[1] Torkild Visnes,[1,3] Kirill Mamonov,[1] Florian Ortis,[1] Thomas Lundbäck,[4] Maria Volkova,[1] Tobias Koolmeister,[1] Elisée Wiita,[1] Olga Loseva,[1] Monica Pandey,[1] Evert J. Homan,[1] Carlos Benítez-Buelga,[1,5] Jonathan Davies,[2] Martin Scobie,[1,6] Ulrika Warpman Berglund,[1,6] Christina Kalderén,[1,6] Pål Stenmark,[2,7] Thomas Helleday,[1,8] Maurice Michel[1,*]

AUTHOR ADDRESS

1 Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

2 Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

3 Department of Biotechnology and Nanomedicine, SINTEF Industry, Trondheim, Norway

4 Chemical Biology Consortium Sweden (CBCS), Science for Life Laboratory, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

5 Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

6 Oxcia AB, Stockholm, Sweden

7 Department of Experimental Medical Science, Lund University, Lund, Sweden

8 Sheffield Cancer Centre, Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

**Target engagement assays**

Differential scanning fluorimetry (DSF) was essentially performed as described here.[1]
CETSA was performed as described here.[2] In brief, HL60 cells were plated to reach 70-80% confluence. The following day, cells were treated with DMSO or compound (20 µM) for 2 h at 37 °C. Cells were then washed, trypsinized and resuspended in growth media. 8 µL cells were aliquoted into PCR tubes and subjected to heating a gradient of 37-62°C over 3 minutes. Samples were then lysed using RIPA buffer, centrifuged and the lysate was collected and stored at −80 °C, until further analysis by western blot.

**Biochemical assays**

The biochemical assay for NEIL1 was performed as described here.[1] For all others enzymes the biochemical assays were based on the publication by Visnes *et al.*[3] and EUbOPEN protocols (https://www.eubopen.org/protocols-reagents).

**Cell culture**

Adherent and suspension cell lines were cultured in RPMI (61870-010 Thermo Fisher Scientific), McCoy's (36600-021 Thermo Fisher Scientific) or DMEM (10566-016 Thermo Fisher Scientific) media depending on the cell line. The media was supplemented with 10% fetal bovine serum (10500064, Thermo Fisher Scientific) and 100 U/ml Penicillin Streptomycin (15140122, Thermo Fisher Scientific) and the cells were cultured at 37°C and 5% carbon dioxide. The BJ-Tert and BJ-Ras cell lines were provided by W. Hahn (Dana-Farber Cancer Institute), MEF *Ogg1*$^{-/-}$ cells from M. Bignami (Istituto Superiore di Sanità, Rome, Italy), HCT116 and HCT116+Chr3 human colon carcinoma cells were obtained from Dr. Bert Vogelstein (2001, Johns Hopkins, Baltimore, MD, USA), Hec59 and Hec59+Chr2, LCL#1 and LCL#2 from J. Benitez (Spanish National Cancer Research Centre, Madrid, Spain), and the rest of the cell lines were sourced from commercial suppliers American Type Culture Collection (ATCC) or the German Collection of Microorganisms and Cell Cultures GmbH (DMSZ). All cultures were passaged a maximum of 25 times after thawing from stock vials and checked for mycoplasma contamination using MycoAlert™ Mycoplasma Detection Kit (Lonza) every other month.

**Cell viability assay**

Cells were seeded in 96- or 384-well plates and incubated for 3 days for combination experiments or 5 days for single-drug exposure experiments. Resazurin (R7017, SigmaAldrich) was added to a final concentration of 0.01 mg/ml resazurin and fluorescence was measured at ex530/em590 after incubation for 2, 4 or 6 h. Curves were fitted using XLfit software (IDBS) or Prism 8.0 (Graphpad Software), and EC50 values were determined.

***In silico* experiments**

Protein preparation: PDB files were imported to Maestro Suite (Schrödinger 2019-3 and 2021-1) and prepared using the Protein Preparation Wizard. In brief, bond orders were assigned, hydrogens were added, disulphide bonds were generated, missing side chains and loops were filled using Prime and het states were generated using Epik for pH 7.0 ± 2.0. Afterwards, the structure was manually fixed upon problem identification. H-bond assignments were performed, waters removed beyond a 3.0 Å radius of het groups and a restrained minimization was performed using the OPLS3e force field, converging the heavy atoms to an RMSD of 0.30 Å. For OGG1 in complex with DNA and flipped-out 8-oxoG (PDB: 1EBM) the mutated Gln249 was changed to lysine, and the DNA was deleted before preparing the structure for docking.

Ligand preparation: structures were exported as sdf from ChemDraw and imported into the Maestro Suite. Using the OPLS3e force field, possible states at a pH 7.0 ±2.0 were generated using Epik. Specific chiralities were retained and a maximum of 32 species per ligand were kept.

Homology modelling: homology modelling was performed using Prime by building a knowledge-based model based on the FASTA sequence of the protein. Gaps were filled, rotamers retained, side chains optimized and the protein preparation wizard rerun on the crude model as described above.

Ligand docking: The docking grid was generated using the prepared structure of PDB:1EBM. F319 was chosen as the centre of a 10 Å x 10 Å x 10 Å box for ligand docking. No other restrictions were made. The compound was docked using the standard docking protocol (Glide SP) without restrictions.

Molecular dynamics: molecular dynamics studies were performed with Desmond as implemented in Schrödinger Suite 2019-3. The function system builder was performed using SPC, a minimized orthorhombic box shape, structure neutralization adding Chloride ions, addition of 0.15M sodium chloride and the OPLS3 force field. Molecular dynamics simulation was then performed using the generated system, with a simulation time of 500 ns, ensemble class NPT, a temperature of 310 K and a pressure of 1.01325 bar. The system was relaxed before simulation.

**Protein crystallization**

Aliquots of purified mOGG1 (22 mg/mL) were pre-incubated with 6.25 mM TH8535 or 12.5 mM TH9525. All protein samples were crystallized via sitting drop vapor diffusion at 18 °C in 0.12 M Ethylene Glycol, 0.1 M Buffer System 2 pH 7.5, 30.0 % (v/v) GOL_P4K (mOGG1-TH8535) or 0.12 M Monosaccharides, 0.1 M Buffer System 2 pH 7.5, 50 % (v/v) Precipitant Mix3 (mOGG1-TH9525), Morpheus Screen (Molecular Dimensions). Protein crystals were fished without additional cryoprotectant and flash frozen in liquid nitrogen.

**Data collection, structure determination, and refinement**

Data for mOGG1-TH8535 was collected at BioMAX (Lund, Sweden) equipped with a EIGER X 16M detector. Data for mOGG1-TH9525 was collected at station I04 of the Diamond Light Source (Oxford, UK) equipped with a PILATUS-6M detector. Complete datasets were collected on single crystals at 100 K for each complex. All datasets were processed and scaled with DIALS[4] and Aimless[5] within the CCP4 suite[6]. Molecular replacement was performed in Phaser[7] using the structure of mouse OGG1 (PDB ID: 6G3Y) with all ligands and waters removed, as the search model. Several rounds of manual model building and refinement were performed using COOT[8] and REFMAC5[9] during which waters and ligands were incorporated into the structures. Data processing and refinement statistics are listed in Table 1. The coordinates and structure factors for mOGG1-TH8535 and mOGG1-TH9525 were deposited in the PDB under codes 7PZ1 and 6G40, respectively.

**Table S1: X-ray crystallography data collection and refinement statistics**

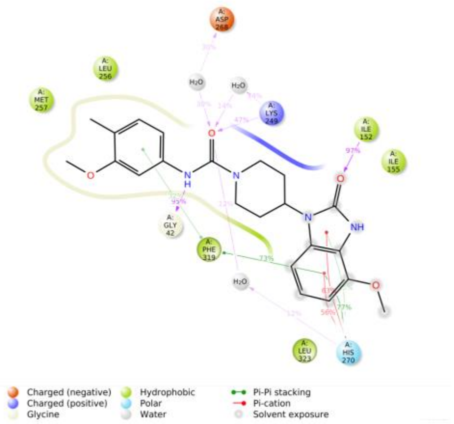
| | **mOGG1-TH8535** | **mOGG1-TH9525** |
|---|---|---|
| **Data collection** | | |
| PDB code | 7PZ1 | 6G40 |
| Station | MAXIV-BioMAX | DLS-I04 |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ |
| Cell dimensions: | | |
| a, b, c (Å) | 81.2, 81.8, 169.6 | 80.9, 81.3, 168.6 |
| α, β, γ (°) | 90, 90, 90 | 90, 90, 90 |

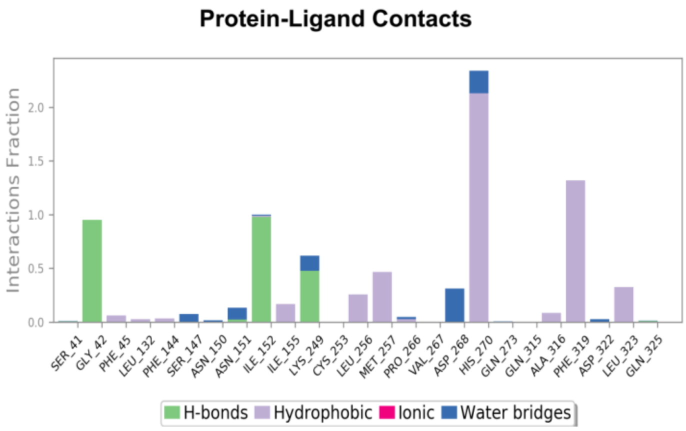
| | | |
|---|---|---|
| Resolution (Å) | 2.45-84.8 (2.45-2.54)[a] | 2.49-81.3 (2.49-2.55)[a] |
| Total reflections | 567,488 | 516,129 |
| Unique reflections | 42,365 | 39,789 |
| $R_{merge}$ | 0.080 (3.49)[a] | 0.077 (0.95)[a] |
| $R_{pim}$ | 0.032 (1.40)[a] | 0.032 (0.40)[a] |
| $CC_{1/2}$ (%) | 0.99 (0.56)[a] | 0.99 (0.53)[a] |
| $I/\sigma$ | 15.5 (1.1)[a] | 17.2 (2.3)[a] |
| Completeness | 100 (100)[a] | 100 (100)[a] |
| Redundancy | 13.4 (13.7)[a] | 13.0 (12.8)[a] |
| **Refinement** | | |
| $R_{work}/R_{free}$ (%) | 25.3/29.0 | 22.0/26.3 |
| *B*-factors: | | |
| Protein (all atoms)[b] | 82.6/91.9/103.5 | 75.5/87.5/92.1 |
| Ligand | 99.2 | 66.9 |
| Water | 78.1 | 66.2 |
| R.m.s. deviations: | | |
| Bond lengths (Å) | 0.002 | 0.007 |
| Bond angles (°) | 1.18 | 1.14 |
| Ramachandran statistics: | | |
| Favoured (%) | 99.9 | 98.9 |
| Outliers (%) | 0.10 | 0.11 |

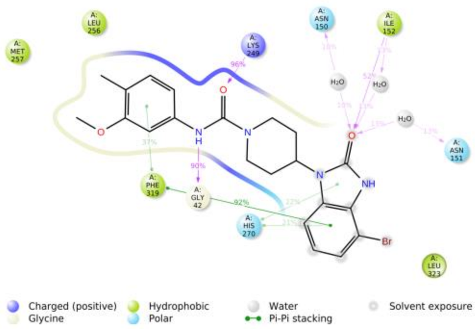
[a] Values in parentheses are for highest-resolution shell. [b] Values for each molecule of the asymmetric unit

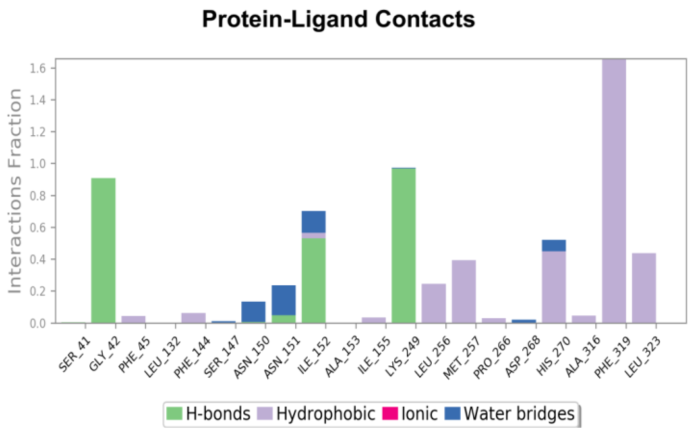
**DNA oligos and Proteins**

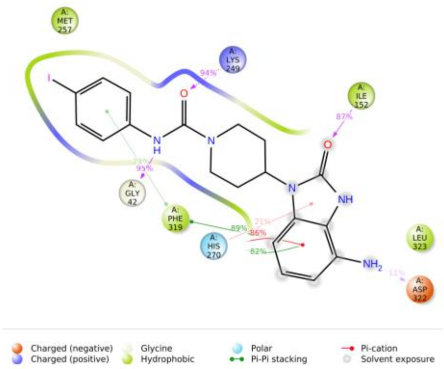
DNA oligos and Proteins were used as reported under https://www.eubopen.org/protocols-reagents , protocol DNA glycosylases. DNA Oligos were ordered from ATD Bio and Protein produced as reported before.[1,3]

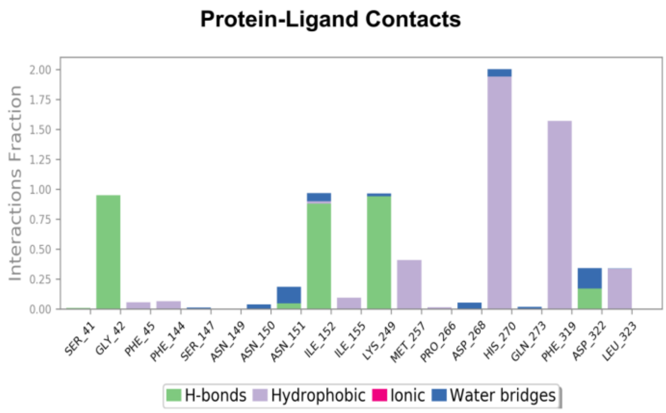

Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Polar
Water
Pi-Pi stacking
Pi-cation
Solvent exposure

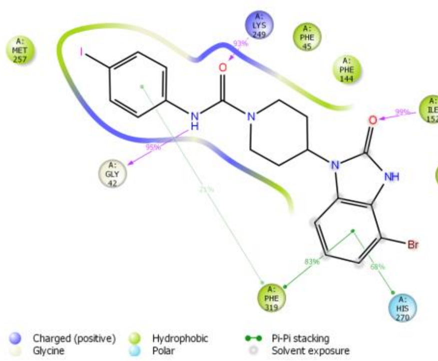

Protein-Ligand Contacts
Interactions Fraction
SER_41 GLY_42 PHE_45 LEU_132 PHE_144 SER_147 ASN_150 ASN_151 ILE_152 ILE_155 LYS_249 CYS_253 LEU_256 MET_257 PRO_266 VAL_267 ASP_268 HIS_270 GLN_273 GLN_315 ALA_316 PHE_319 ASP_322 LEU_323 GLN_325
H-bonds Hydrophobic Ionic Water bridges


Charged (positive)
Glycine
Hydrophobic
Polar
Water
Pi-Pi stacking
Solvent exposure


Protein-Ligand Contacts
Interactions Fraction
SER_41 GLY_42 PHE_45 LEU_132 PHE_144 SER_147 ASN_150 ASN_151 ILE_152 ALA_153 ILE_155 LYS_249 LEU_256 MET_257 PRO_266 ASP_268 HIS_270 ALA_316 PHE_319 LEU_323
H-bonds Hydrophobic Ionic Water bridges


Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Polar
Pi-Pi stacking
Pi-cation
Solvent exposure


Protein-Ligand Contacts
Interactions Fraction
SER_41 GLY_42 PHE_45 PHE_144 SER_147 ASN_149 ASN_150 ASN_151 ILE_152 ILE_155 LYS_249 MET_257 PRO_266 ASP_268 HIS_270 GLN_273 PHE_319 ASP_322 LEU_323
H-bonds Hydrophobic Ionic Water bridges


Charged (positive)
Glycine
Hydrophobic
Polar
Pi-Pi stacking
Solvent exposure

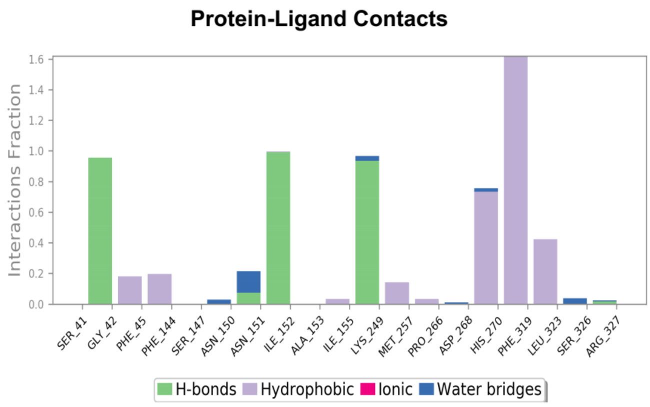

Protein-Ligand Contacts
Interactions Fraction
SER_41 GLY_42 PHE_45 PHE_144 SER_147 ASN_150 ASN_151 ILE_152 ALA_153 ILE_155 LYS_249 MET_257 PRO_266 ASP_268 HIS_270 PHE_319 LEU_323 SER_326 ARG_327
H-bonds Hydrophobic Ionic Water bridges

**Figure S1: Results of Molecular Dynamics simulation of 500 ns of prepared complexes of TH9525, TH8535, TH5675 and TH5487.** Left panels: Percentage of interaction time within 500 ns simulation is given for each position of the inhibitors and their respective binding OGG1 residues and water molecules. Right panels: Fraction of contacts for the same time frame are shown.

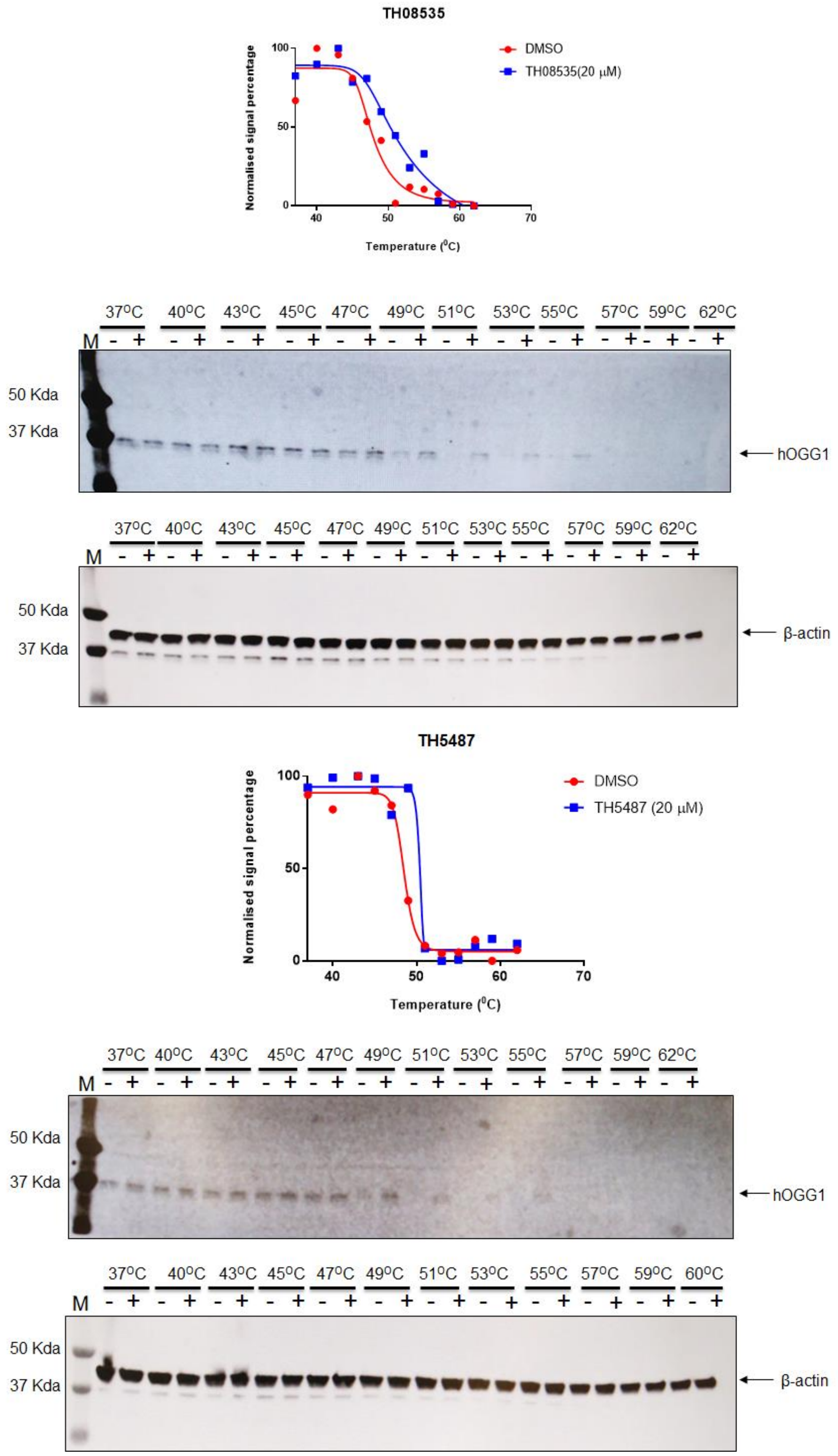
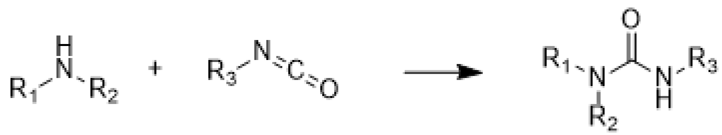


**Figure S2: Cellular thermal shift assay of TH8535 and TH5487 in HL60 cells confirms target engagement:** Upper panel: TH8535 engages OGG1. β-actin as control. Lower panel: TH5487 engages OGG1. Experimental details see Method section.

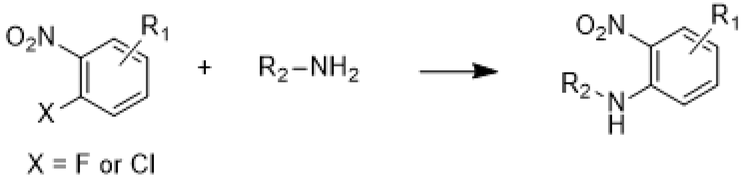
**Synthetic Section**

**General Information**. All reagents and solvents were purchased from commercial vendors and used without further purification. Unless otherwise stated, reactions were performed without care to exclude air or moisture. Analytical thin-layer chromatography was performed on silica gel 60 F-254 plates (E. Merck) and visualized under a UV lamp. Flash column chromatography was performed in a Biotage® SP1 MPLC system using Fisher Chemical silica gel 60Å. $^1$H and $^{13}$C NMR spectra were recorded on Bruker DRX-400 MHz or Bruker Ascend 400 MHz spectrometers. Chemical shifts are expressed in parts per million (ppm) and referenced to the residual solvent peak. Analytical LC-MS were performed either on an Agilent MSD mass spectrometer connected to an Agilent 1100 system or an Agilent 1260 infinity II with a G6125B mass spectrometer. Columns used were ACE 3 C8 (50 x 3.0 mm); $H_2O$ (+ 0.1% TFA) and MeCN were used as mobile phases at a flow rate of 1 mL/min, or Xterra MSC18 (50 x 3.0 mm) column where $H_2O$ (containing 10 mM $NH_4HCO_3$; pH = 10) and MeCN were used as mobile phases at a flow rate of 1 mL/min. For LC-MS, detection was made by UV and MS (ESI+). Preparative LC was performed on a Gilson system using Waters C18 OBD 5 µm column (30x75 mm) with water buffer (50 mM $NH_4HCO_3$ at pH 10) and acetonitrile as mobile phases using a flow rate of 45 mL/min. All final compounds were assessed to be >95% pure by LC-MS analysis.

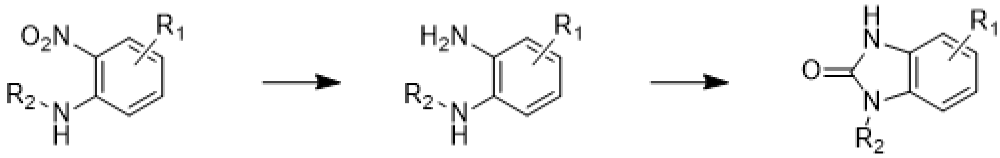
**General procedures**



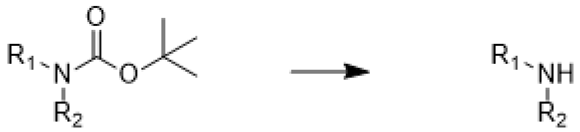

**General procedure A**: To the corresponding amine (0.10 mmol) in DCM (2 mL) was added a suitable isocyanate (0.10 mmol) dissolved in DCM (1.0 mL). The resulting mixture was stirred at 20 °C for 3 – 16 h. If the corresponding amine used was in the form of an ammonium salt, then triethylamine (0.1 mmol) was added to the reaction mixture. After complete reaction the mixture was purified by silica gel chromatography or by preparative liquid chromatography.



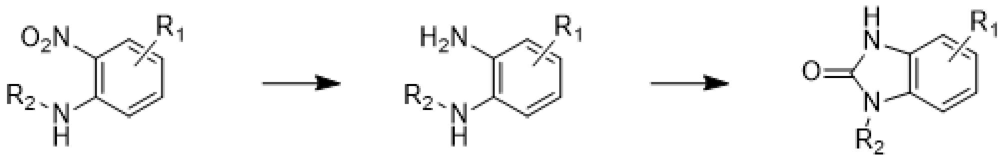
**General procedure B**: A mixture of the corresponding 2-fluoro- or 2-chloro-1-nitrobenzene compound (1.0 equiv.), a suitable amine (1.1 equiv.), and *N*,*N*-diisopropylethylamine (1.2 equiv.) was stirred in 2-propanol (0.2 M) at 120 °C for 12 – 72 h in a sealed vial. Thereafter, the mixture was poured into $NaHCO_3$ and extracted with DCM×3. The combined organics were dried with $MgSO_4$, filtered, concentrated, and purified by silica gel chromatography.



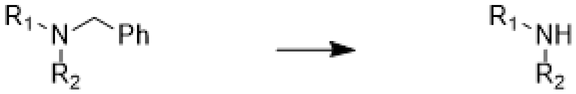
**General procedure C**: A two necked round bottle fitted with a thermometer was charged with a mixture of a substituted 2-amino-1-nitrobenzene compound (1.0 equiv.) and $NiCl_2$ (0.20 equiv.), then acetonitrile/water (9:1 v/v, 0.05 – 0.1 M) was added followed by portion wise addition $NaBH_4$ (4.0 equiv.) at such rate that the temperature did not exceed 35 °C. After complete reaction, DCM was added, and the liquids were poured into $NaHCO_3$ by means of decantation to avoid the black residues. The aqueous phase was extracted with DCM×3 and the combined extracts were dried with $MgSO_4$ and filtered. To the filtrate was then added *N*,*N*-diisopropylethylamine (2.2 equiv.) and diphosgene (0.50 equiv.) or triphosgene (0.34 equiv.). After complete reaction, the mixture was concentrated and purified by silica gel chromatography.



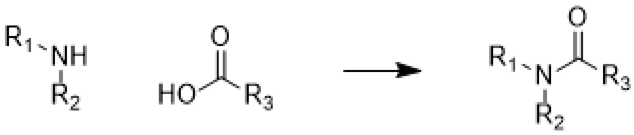
**General procedure D**: The corresponding tert-butyl carbamate compound was dissolved in DCM, then trifluoroacetic acid (5 – 15 equiv.) was added and the mixture was stirred at 20 °C for 10 – 60 min. After complete reaction, the solvents were removed by co-evaporation with 2-propanol. Unless otherwise stated, no further purification was done.



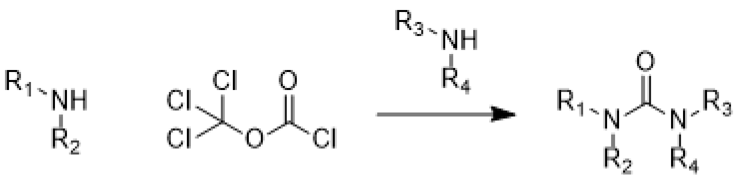
**General procedure E**: A mixture of the corresponding nitrobenzene compound (1.0 equiv.) and Pd/C (0.05 equiv.) was stirred in THF at 20 °C for 12 – 24 h under an $H_2$ atmosphere provided by a balloon. After complete reaction the balloon was removed and N,N-diisopropylethylamine (2.0 equiv.) and triphosgene (0.35 equiv.) were added. The resulting mixture was stirred for 20 – 60 min at 20 °C and was then filtered, concentrated, and purified by silica gel chromatography.



**General procedure F**: A mixture of the corresponding benzyl protected compound (1 equiv.) and Pd/C (0.1 equiv.) was stirred in 1,4-dioxane and cyclohexene (10:1 v/v) in a sealed vial at 120 °C for 2 – 16 h. Upon complete reaction the mixture was filtered, concentrated, and purified by silica gel chromatography.



**General procedure G**: A mixture of the corresponding carboxylic acid (1.0 equiv.), an appropriate amine (2 equiv.), propylphosphonic anhydride (4 equiv.) and *N,N*-diisopropylethylamine (3.0 equiv.) was stirred in THF or acetonitrile at an elevated temperature for 3 – 16 h. After complete reaction the mixture was purified by silica gel chromatography or by preparative liquid chromatography.



**General procedure H**: To a mixture of a suitable amine or a salt thereof (0.10 mmol) and *N,N*-diisopropylethylamine (0.035 mL, 0.20 mmol) in DCM (1.0 mL) was added diphosgene (0.050 mmol) under vigorous stirring. The mixture was stirred at 20 °C for 5 – 15 min after which it was added to a separate mixture of the corresponding amine or a salt thereof (0.10 mmol) and *N,N*-diisopropylethylamine (0.035 mL, 0.20 mmol) in DCM (1.0 mL). The resulting mixture was stirred at 20 °C for 3 – 16 h. After complete reaction the mixture was purified by silica gel chromatography or by preparative liquid chromatography.

Synthetic procedures to yield compounds **1**, **26** and **28** have been reported before.[3,10]

*N-(3-fluorophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**2**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 3-fluorophenyl isocyanate in 63% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.84 (br. s, 1 H), 8.79 (br. s, 1 H), 7.45 – 7.53 (m, 1 H), 7.19 – 7.32 (m, 3 H), 6.95 – 7.03 (m, 3 H), 6.71 – 6.79 (m, 1 H), 4.36 – 4.46 (m, 1 H), 4.26 – 4.35 (m, 2 H), 2.91 – 3.01 (m, 2 H), 2.23 – 2.35 (m, 2 H), 1.70 – 1.80 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 162.7 (d, $^{1}J_{CF}$ = 240 Hz), 154.9, 154.2, 143.2 (d, $^{3}J_{CF}$ = 11 Hz), 130.2 (d, $^{3}J_{CF}$ = 9.2 Hz), 129.7, 128.8, 121.1, 120.8, 115.4, 109.3, 108.9, 108.3 (d, $^{2}J_{CF}$ = 21 Hz), 106.4 (d, $^{2}J_{CF}$ = 26 Hz), 50.5, 44.0, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}FN_4O_2$ 354.1492, found: 354.1580.

*N-(3-chlorophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**3**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 3-chlorophenyl isocyanate in 83% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.85 (br. s, 1 H), 8.77 (br. s, 1 H), 7.67 – 7.71 (m, 1 H), 7.40 – 7.45 (m, 1 H), 7.19 – 7.29 (m, 3 H), 6.95 – 7.00 (m, 3 H), 4.36 – 4.44 (m, 1 H), 4.26 – 4.32 (m, 2 H), 2.91 – 2.99 (m, 2 H), 2.26 – 2.32 (m, 2 H), 1.70 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.2, 142.8, 133.2, 130.4, 129.7, 128.8, 121.6, 121.1, 120.8, 119.2, 118.1, 109.3, 108.9, 50.5, 44.0, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}ClN_4O_2$ 370.1197, found: 370.1287.

*N-(4-methylphenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**4**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-tolyl isocyanate in 80% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.85 (br. s, 1 H), 8.48 (br. s, 1 H), 7.34 – 7.39 (m, 2 H), 7.18 – 7.22 (m, 1 H), 7.02 – 7.07 (m, 2 H), 6.95 – 7.00 (m, 3 H), 4.34 – 4.43 (m, 1 H), 7.25 – 7.32 (m, 2 H), 2.88 – 2.96 (m, 2 H), 2.21 – 2.32 (m, 5 H), 1.68 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.4, 154.2, 138.5, 130.9, 129.7, 129.2, 128.8, 121.0, 120.8, 120.2, 109.3, 108.9, 50.58697, 44.0, 29.2, 20.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{22}N_4O_2$ 350.1743, found: 350.1851.

*N-(4-fluorophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide* ***(5)***.

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-fluorophenyl isocyanate in 74% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.85 (br. s, 1 H), 8.61 (br. s, 1 H), 7.46 – 7.52 (m, 2 H), 7.18 – 7.22 (m, 1 H), 7.05 – 7.11 (m, 2 H), 6.95 – 7.00 (m, 3 H), 4.35 – 4.43 (m, 1 H), 4.26 – 4.32 (m, 2 H), 2.89 – 2.97 (m, 2 H), 2.23 – 2.32 (m, 2 H), 1.70 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 157.8 (d, $^1J_{CF}$ = 238 Hz), 155.3, 154.2, 137.4, 129.7, 128.8, 121.8 (d, $^3J_{CF}$ = 7.2 Hz), 121.0, 120.8, 115.2 (d, $^2J_{CF}$ = 22 Hz), 109.3, 108.9, 50.6, 44.0, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}FN_4O_2$ 354.1492, found: 354.1596.

*N-(4-chlorophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide* ***(6)***.

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-chlorophenyl isocyanate in 81% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.84 (br. s, 1 H), 8.71 (br. s, 1 H), 7.50 – 7.56 (m, 2 H), 7.18 – 7.23 (m, 1 H), 6.95 – 7.00 (m, 3 H), 4.36 – 4.43 (m, 1 H), 4.26 – 4.32 (m, 2 H), 2.90 – 2.98 (m, 2 H), 2.23 – 2.33 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.0, 154.2, 140.2, 129.7, 128.8, 128.6, 125.6, 121.4, 121.0, 120.8, 109.3, 108.9, 50.5, 44.0, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}ClN_4O_2$ 370.1197, found: 370.1291.

*N-(4-bromophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide* ***(7)***.

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-bromophenyl isocyanate in 95% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.85 (br. s, 1 H), 8.72 (br. s, 1 H), 7.50 (d, J = 9.0 Hz, 2 H), 7.42 (d, J = 9.0 Hz, 2 H), 7.20 – 7.24 (m, 1 H), 6.96 – 7.01 (m, 3 H), 4.40 (tt, J = 12.2, 4.1 Hz, 1 H), 4.26 – 4.34 (m, 2 H), 2.90 – 3.00 (m, 2 H), 2.22 – 2.35 (m, 2 H), 1.70 – 1.78 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.5, 153.7, 140.2, 131.0, 129.3, 128.3, 121.4, 120.6, 120.4, 113.1, 108.8, 108.5, 50.0, 43.5, 28.7. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}BrN_4O_2$ 414.0691, found: 414.0771.

*N-(4-iodophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**8**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-iodophenyl isocyanate in 90% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.83 (br. s, 1 H), 8.68 (br. s, 1 H), 7.54 – 7.58 (m, 2 H), 7.34 – 7.38 (m, 2 H), 7.18 – 7.22 (m, 1 H), 6.95 – 7.00 (m, 2 H), 4.35 – 4.43 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.90 – 2.97 (m, 2 H), 2.23 – 2.32 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.2, 141.2, 137.3, 129.7, 128.8, 122.2, 121.0, 120.8, 109.3, 108.9, 85.0, 50.5, 43.9, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}IN_4O_2$ 462.0553, found: 462.0667.

*N-(3,4-dichlorophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**9**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 3,4-dichlorophenyl isocyanate in 87% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.84 (br. s, 1 H), 8.86 (br. s, 1 H), 7.86 – 7.89 (m, 1 H), 7.45 – 7.50 (m, 2 H), 7.20 – 7.24 (m, 1 H), 6.96 – 6.99 (m, 3 H), 4.37 – 4.44 (m, 1 H), 4.25 – 4.32 (m, 2 H), 2.92 – 3.00 (m, 2 H), 2.23 – 2.32 (m, 2 H), 1.71 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.7, 154.2, 141.5, 131.0, 130.6, 129.7, 128.7, 123.3, 121.1, 120.8, 120.8, 119.7, 109.3, 108.9, 50.4, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}Cl_2N_4O_2$ 404.0807, found: 404.0900.

*N-(4-nitrophenyl)-4-(2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxamide (**10**).*

This compound was prepared according to general procedure A from 1-piperidin-4-yl-1,3-dihydro-2h-benzimidazol-2-one and 4-nitrophenyl isocyanate in 68% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.85 (br. s, 1 H), 9.30 (br. s, 1 H), 8.14 – 8.24 (m, 2 H), 7.72 – 7.78 (m, 2 H), 7.20 – 7.25 (m, 1 H), 6.96 – 7.00 (m, 3 H), 4.38 – 4.46 (m, 1 H), 4.29 – 4.35 (m, 2 H), 2.96 – 3.04 (m, 2 H), 2.26 – 2.35 (m, 2 H), 1.73 – 1.79 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 154.2, 148.1, 141.2, 129.7, 128.8, 125.2, 121.1, 120.8, 118.8, 109.3, 109.0, 50.4, 44.1, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}N_5O_4$ 381.1437, found: 381.1525.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-phenylpiperidine-1-carboxamide (**11**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and phenyl isocyanate in 88% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.03 (br. s, 1 H), 8.56 (br. s, 1 H), 7.45 – 7.50 (m, 2 H), 7.20 – 7.26 (m, 3 H), 6.98 – 7.04 (m, 2 H), 6.90 – 6.95 (m, 1 H), 4.34 – 4.42 (m, 1 H), 4.26 – 4.32 (m, 2 H), 2.88 – 2.96 (m, 2 H), 2.20 – 2.30 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.2, 154.1, 141.1, 130.0, 128.7, 125.3, 122.1, 120.5, 120.1, 120.0, 110.1, 109.1, 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{19}ClN_4O_2$ 370.1197, found: 370.1300.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3-fluorophenyl)piperidine-1-carboxamide (**12**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 3-fluorophenyl isocyanate in 65% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.03 (br. s, 1 H), 8.78 (br. s, 1 H), 7.45 – 7.50 (m, 1 H), 7.23 – 7.29 (m, 3 H), 6.99 – 7.04 (m, 2 H), 6.71 – 6.76 (m, 1 H), 4.35 – 4.43 (m, 1 H), 4.27 – 4.32 (m, 2 H), 2.90 – 2.98 (m, 2 H), 2.20 – 2.29 (m, 2 H), 1.72 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 162.6 (d, $^{1}J_{CF}$ = 240 Hz), 154.8, 154.1, 143.1 (d, $^{3}J_{CF}$ = 11 Hz), 130.2 (d, $^{3}J_{CF}$ = 9.3 Hz), 130.0, 128.7, 125.3, 120.5, 115.4, 110.1, 109.1, 108.3 (d, $^{2}J_{CF}$ = 21 Hz), 106.4 (d, $^{2}J_{CF}$ = 26 Hz), 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}ClFN_4O_2$ 388.1102, found: 388.1204.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3-chlorophenyl)piperidine-1-carboxamide (**13**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 3-chlorophenyl isocyanate in 75% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.76 (br. s, 1 H), 7.68 – 7.70 (m, 1H), 7.41 – 7.44 (m, 2 H), 7.23 – 7.28 (m, 2 H), 6.96 – 7.04 (m, 3 H), 4.36 – 4.43 (m, 1 H), 4.26 – 4.32 (m, 2 H), 2.91 – 2.98 (m, 2 H), 2.20 – 2.29 (m, 2 H), 1.72 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.8, 154.1, 142.8, 133.2, 130.4, 130. 0, 128.7, 125.3, 121.6, 120.5, 119.2, 118.1, 110.1, 109.1, 50.7, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}Cl_2N_4O_2$ 404.0807, found: 404.0900.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-methylphenyl)piperidine-1-carboxamide (**14**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 4-tolyl isocyanate in 78% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.47 (br. s, 1 H), 7.33 – 7.38 (m, 2 H), 7.21 – 7.25 (m, 1 H), 6.98 – 7.06 (m, 4 H), 4.33 – 4.41 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.87 – 2.94 (m, 2 H), 2.19 – 2.28 (m, 5 H), 1.69 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.3, 154.1, 138.5, 130.9, 130.0, 129.2, 128.8, 125.3, 120.5, 120.2, 110.1, 109.1, 50.9, 43.9, 29.1, 20.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}ClN_4O_2$ 384.1353, found: 384.1473.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-fluorophenyl)piperidine-1-carboxamide (**15**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 4-fluorophenyl isocyanate in 70% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.61 (br. s, 1 H), 7.44 – 7.51 (m, 2 H), 7.20 – 7.26 (m, 1 H), 6.96 – 7.10 (m, 4 H), 4.34 – 4.43 (m, 1 H), 4.24 – 4.32 (m, 2 H), 2.87 – 2.96 (m, 2 H), 2.19 – 2.29 (m, 2 H), 1.68 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 157.8 (d, $^{1}J_{CF}$ = 238 Hz), 155.2, 154.1, 137.4, 130.0, 128.7, 125.3, 121.8 (d, $^{3}J_{CF}$ = 6.7 Hz), 120.5, 115.2 (d, $^{2}J_{CF}$ = 22 Hz), 110.1, 109.1, 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}ClFN_4O_2$ 388.1102, found: 388.1212.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-chlorophenyl)piperidine-1-carboxamide (**16**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 4-chlorophenyl isocyanate in 85% yield. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.71 (br. s, 1 H), 7.50 – 7.55 (m, 2 H), 7.22 – 7.30 (m, 3 H), 6.98 – 7.04 (m, 2 H), 4.34 – 4.42 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.90 – 2.97 (m, 2 H), 2.19 – 2.29 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.1, 140.2, 130.0, 128.7, 128.6, 125.6, 125.3, 121.4, 120.5, 110.1, 109.1, 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}Cl_2N_4O_2$ 404.0807, found: 404.0900.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-bromophenyl)piperidine-1-carboxamide (**17**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 4-bromophenyl isocyanate in 88% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.71 (br. s, 1 H), 7.46 – 7.50 (m, 2 H), 7.39 – 7.43 (m, 2 H), 7.23 – 7.26 (m, 1 H), 6.99 – 7.04 (m, 2 H), 4.35 – 4.42 (m, 1 H), 4.26 – 4.31 (m, 2 H), 2.89 – 2.97 (m, 2 H), 2.20 – 2.29 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.1, 140.6, 131.5, 130.0, 128.7, 125.3, 121.8, 120.5, 113.6, 110.1, 109.1, 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}BrClN_4O_2$ 448.0302, found: 448.0396.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**18**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 4-iodophenyl isocyanate in 90% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.68 (br. s, 1 H), 7.54 – 7.58 (m, 2 H), 7.33 – 7.38 (m, 2 H), 7.22 – 7.26 (m, 1 H), 6.99 – 7.04 (m, 2 H), 4.34 – 4.41 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.89 – 2.96 (m, 2 H), 2.19 – 2.28 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.1, 141.1, 137.3, 130.0, 128.7, 125.3, 122.2, 120.5, 110.1, 109.1, 85.0, 50.8, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}ClIN_4O_2$ 496.0163, found: 496.0258.

*4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3,4-dichlorophenyl)piperidine-1-carboxamide (**19**).*

This compound was prepared according to general procedure A from 5-Chloro-1-(4-piperidyl)-2-benzimidazolinone and 3,4-dichlorophenyl isocyanate in 86% yield. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.86 (br. s, 1 H), 7.86 – 7.89 (m, 1 H), 7.47 – 7.49 (m, 2 H), 7.23 – 7.27 (m, 1 H), 6.98 – 7.03 (m, 2 H), 4.35 – 4.43 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.91 – 2.98 (m, 2 H), 2.20 – 2.29 (m, 2 H), 1.70 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.6, 154.1, 141.5, 131.0, 130.6, 130.0, 128.7, 125.3, 123.3, 120.8, 120.5, 119.7, 110.1, 109.1, 50.7, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{17}Cl_3N_4O_2$ 438.0417, found: 438.0505.

*4-(4-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**20**).*

Step 1: tert-butyl 4-(3-methyl-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure B from 1-fluoro-3-methyl-2-nitrobenzene (310 mg, 2.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (400 mg, 2.0 mmol). Yield 48%. LCMS $[M+H]^+$ 336.

Step 2: (tert-butyl 4-(4-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure E from tert-butyl 4-(3-methyl-2-nitro-anilino)piperidine-1-carboxylate (320 mg, 0.96 mmol). Yield 95%. LCMS $[M\text{-isobutene}+H]^+$ 276. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.91 (s, 1 H), 7.01 (d, $J$ = 7.8 Hz, 1 H), 6.89 (t, $J$ = 7.8 Hz, 1 H), 6.79 (d, $J$ = 7.8 Hz, 1 H), 4.32 (tt, $J$ = 12.2, 4.1 Hz, 1 H), 4.04 – 4.15 (m, 2 H), 2.76 – 2.96 (m, 2 H), 2.26 (s, 3 H), 2.15 – 2.34 (m, 2 H), 1.65 – 1.69 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.4, 154.3, 129.3, 127.6, 122.2, 120.8, 119.0, 106.5, 79.3, 50.4, 43.9 (br. s), 43.1 (br. s), 29.0 (br. s), 28.6, 16.6.

Step 3: 4-methyl-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure D from tert-butyl 4-(4-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (300 mg, 0.91 mmol). Yield: quant. LCMS $[M+H]^+$ 232. 1H-NMR (400 MHz, DMSO-d6) δ (ppm) 11.00 (br. s., 1 H), 8.71 (br. s, 1 H), 8.47 (br. s, 1 H), 7.11 - 7.18 (m, 1 H), 6.90 - 6.97 (m, 1 H), 6.80 - 6.86 (m, 1 H), 4.46 - 4.57 (m, 1 H), 3.38 - 3.49 (m, 2 H), 3.03 - 3.18 (m, 2 H), 2.53 - 2.63 (m, 2 H), 2.28 (s, 3 H), 1.80 - 1.91 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 4-methyl-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 87%. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.91 (br. s, 1 H), 8.69 (br. s, 1 H), 7.54 – 7.58 (m, 2 H), 7.34 – 7.38 (m, 2 H), 7.03 (br. d, $^3J$ = 7.3 Hz, 1 H), 6.89 (dt, $^3J$ = 7.7, 2.2 Hz, 1 H), 6.79 (br. d, $^3J$ = 7.0 Hz, 1 H), 4.34 – 4.41 (m, 1 H), 4.25 – 4.31 (m, 2 H), 2.89 – 2.97 (m, 2 H), 2.23 – 2.32 (m, 5 H), 1.69 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.4, 141.2, 137.3, 129.4, 127.6, 122.2, 122.1, 120.8, 119.0, 106.5, 85.0, 50.5, 44.0, 29.2, 16.6. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}IN_4O_2$ 476.0709, found: 476.0824.

*4-(5-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**21**).*

Step 1: tert-butyl 4-[(4-methyl-2-nitrophenyl)amino]piperidine-1-carboxylate was synthesized according to General procedure B from 1-fluoro-4-methyl-2-nitrobenzene (160 mg, 1.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (200 mg, 1.0 mmol). Yield 79%. LCMS $[M+H]^+$ 336.

Step 2: tert-butyl 4-(5-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure C from tert-butyl 4-[(4-methyl-2-nitrophenyl)amino]piperidine-1-carboxylate (260 mg 0.77 mmol). Yield: 80%. LCMS $[M+H]^+$ 332. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.73 (s, 1 H), 7.04 – 7.06 (m, 1 H), 6.77 – 6.80 (m, 2 H), 4.29 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.03 – 4.14 (m, 2 H), 2.77 – 2.96 (m, 2 H), 2.29 (s, 3 H), 2.13 – 2.21 (m, 2 H), 1.63 – 1.69 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 154.2, 130.1, 128.9, 127.5, 121.3, 109.8, 108.7, 79.3, 50.3, 43.9 (br. s), 43.1 (br. s), 29.1 (br. s), 28.6, 21.3.

Step 3: 5-Methyl-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure D from tert-butyl 4-(5-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (200 mg, 0.62 mmol). Yield: quant. LCMS $[M+H]^+$ 232. 1H-NMR (400 MHz, DMSO-d6) δ ppm 10.83 (s, 1 H), 8.75 (br. s., 1 H), 8.44 - 8.60 (m, 1 H), 7.19 (d, *J* =8.5 Hz, 1 H), 6.79 - 6.86 (m, 2 H), 4.45 - 4.55 (m overlap w water, 1 H), 3.43 (m, 2 H), 3.10 (m, 2 H), 2.46 - 2.59 (m overlap w DMSO, 2 H), 2.30 (s, 3 H), 1.84 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 5-methyl-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 90%. LCMS $[M+H]^+$ 477. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.73 (br. s, 1 H), 8.68 (br. s, 1 H), 7.53 – 7.58 (m, 2 H), 7.33 – 7.38 (m, 2 H), 7.05 – 7.09 (m, 1 H), 6.76 – 6.80 (m, 2 H), 4.31 – 4.39 (m, 1 H), 4.24 – 4.31 (m, 2 H), 2.88 – 2.96 (m, 2 H), 2.19- 2.30 (m, 5 H), 1.67 – 1.73 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.3, 141.2, 137.3, 130.1, 128.9, 127.5, 122.2, 121.3, 109.8, 108.7, 85.0, 50.4, 44.0, 29.2, 21.3. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}IN_4O_2$ 476.0709, found: 476.0833.

*4-(6-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**22**).*

Step 1: tert-butyl 4-(5-methyl-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure B from 2-fluoro-4-methyl-1-nitrobenzene (310 mg, 2.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (400 mg, 2.0 mmol). Yield 81%. LCMS $[M+H]^+$ 336.

Step 2: tert-butyl 4-(6-methyl-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure E from tert-butyl 4-(5-methyl-2-nitro-anilino)piperidine-1-carboxylate (540 mg, 1.62 mmol). Yield: 89%. LCMS $[M\text{-isobutene}]^+$

276. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.69 (s, 1 H), 7.00 (br. s, 1 H), 6.85 (d, *J* = 7.8 Hz, 1 H), 6.78 (br. d, *J* = 7.8 Hz, 1 H), 4.31 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.04 – 4.13 (m, 2 H), 2.77 – 2.96 (m, 2 H), 2.31 (s, 3 H), 2.16 – 2.24 (m, 2 H), 1.63 – 1.68 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.4, 154.3, 129.9, 126.5, 121.5, 109.4, 109.0, 79.3, 50.2, 43.9 (br. s), 43.2 (br. s), 28.9 (br. s), 28.6, 21.6.

Step 3: 6-methyl-3-(4-piperidyl)-1H-benzimidazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure D from tert-butyl 4-(6-methyl-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate (470 mg, 1.4 mmol). Yield: quant. LCMS $[M+H]^+$ 232. $^1$H NMR (400 MHz, DMSO-$d_6$) □ ppm 10.79 (s, 1 H), 8.57 - 8.68 (m, 1 H), 8.36 - 8.50 (m, 1 H), 7.12 (s, 1 H), 6.88 (d, *J* = 8.0 Hz, 1 H), 6.82 (ddd, *J* = 8.0, 1.4, 0.6 Hz, 1 H), 4.50 (tt, *J* = 12.2, 4.5 Hz, 1 H), 3.39 - 3.48 (m, 3 H), 3.03 - 3.16 (m, 2 H), 2.52 - 2.61 (m, 2 H), 2.35 (s, 3 H), 1.80 - 1.90 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 6-methyl-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 85%. LCMS $[M+H]^+$ 477. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.70 (br. s, 1 H), 8.70 (br. s, 1 H), 7.54 – 7.58 (m, 2 H), 7.34 – 7.38 (m, 2 H), 7.04 (br. s, 1H), 6.85 (br. d, *J* = 7.9 Hz, 1 H), 6.78 (br. d, *J* = 7.9 Hz, 1 H), 4.36 (tt, *J* = 12.2, 4.0 Hz, 1 H), 4.25 – 4.31 (m, 2 H), 2.89 – 2.96 (m, 2 H), 2.24 – 2.31 (m, 5 H), 1.68 – 1.74 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.0, 154.3, 141.2, 137.4, 129.9, 129.9, 126.5, 122.2, 121.5, 109.4, 109.0, 85.0, 50.5, 44.0, 29.1, 21.6. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}IN_4O_2$ 476.0709, found: 476.0830.

*4-(7-methyl-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**23**).*

Step 1: tert-butyl 4-(2-methyl-6-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure B from 2-fluoro-1-methyl-3-nitro-benzene (310 mg, 2.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (400 mg, 2.0 mmol). Yield: 74%. LCMS $[M+H]^+$ 336.

Step 2: tert-butyl 4-(7-methyl-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure E from tert-butyl 4-(2-methyl-6-nitro-anilino)piperidine-1-carboxylate (500 mg, 1.5 mmol). Yield 75%. LCMS $[M\text{-isobutene}+H]^+$ 276. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.76 (s, 1 H), 6.86 (t, *J* = 7.6 Hz, 1 H), 6.79 (d, *J* = 7.6 Hz, 1 H), 6.75 (t, *J* = 7.6 Hz, 1 H), 4.45 – 4.51 (m, 1 H), 4.01 – 4.12 (m, 2 H), 2.72

– 2.92 (m, 2 H), 2.55 (s, 3 H), 2.41 – 2.50 (m, 2 H), 1.71 – 1.76 (m, 2 H), 1.43 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.6, 154.2, 129.2, 128.5, 124.7, 121.2, 118.9, 107.4, 79.2, 53.0, 44.1 (br. s), 43.2 (br. s), 29.2 (br. s), 28.6 ,19.8 (br. s).

Step 3: 7-methyl-3-(4-piperidyl)-1H-benzimidazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure D from: tert-butyl 4-(7-methyl-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate (360 mg, 1.1 mmol). Yield: quant. LCMS $[M+H]^+$ 232. $^1$H NMR (400 MHz, DMSO-d6) δ ppm 10.86 (s, 1 H), 8.66 - 8.78 (m, 1 H), 8.31 - 8.45 (m, 1 H), 6.89 (t, *J* =7.5 Hz, 1 H), 6.82 (dd, *J* =7.5, 1.5 Hz, 1 H), 6.77 (ddd, *J* =7.5, 1.5, 0.8 Hz, 1 H), 4.55 - 4.66 (m, 1 H), 3.41 (br. s., 2 H), 3.02 - 3.16 (m, 2 H), 2.76 - 2.89 (m, 2 H), 2.58 (s, 3 H), 1.92 - 2.00 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 7-methyl-3-(4-piperidyl)-1H-benzimidazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 88%. LCMS $[M+H]^+$ 477. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.76 (br. s, 1 H), 8.67 (br. s, 1 H), 7.54 – 7.57 (m, 2 H), 7.34 – 7.57 (m, 2 H), 6.86 (t, $^3J$ = 7.6 Hz, 1 H), 6.80 (d, $^3J$ = 7.6 Hz, 1 H), 6.76 (d, $^3J$ = 7.6 Hz, 1 H), 4.51 – 4.58 (m, 2 H), 4.22 – 4.28 (m, 2 H), 2.85 – 2.92 (m, 2 H), 2.58 (s, 3 H), 2.51 – 2.58 (overlapping m, 2 H), 1.76 – 1.81 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.8, 154.6, 141.2, 137.3, 129.2, 128.5, 124.7, 122.2, 121.2, 119.0, 107.4, 85.0, 53.2, 44.0, 29.4, 19.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}IN_4O_2$ 476.0709, found: 476.0825.

*4-(4-fluoro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**24**).*

Step 1: tert-butyl 4-(3-fluoro-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure B from 1,3-difluoro-2-nitrobenzene (320 mg, 2,0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (400 mg, 2.0 mmol). Yield 62%. LCMS $[M\text{-}isobutene+H]^+$ 284.

Step 2: tert-butyl 4-(4-fluoro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure C from tert-butyl 4-(3-fluoro-2-nitro-anilino)piperidine-1-carboxylate (410 mg, 1.2 mmol). Yield: 80%. LCMS $[M\text{-}isobutene+H]^+$ 280. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.42 (s, 1 H), 7.07 (d, *J* = 8.1 Hz, 1 H), 6.99 (td, *J* = 8.1, 5.2 Hz, 1 H), 6.88 (dd, *J* = 10.1, 8.1 Hz, 1 H), 4.34 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.05 – 4.15 (m, 2 H), 2.74 – 3.00 (m, 2 H), 2.20 (dd, *J* = 12.6, 4.5 Hz, 1 H), 2.16 (dd, *J* = 12.6, 4.5 Hz, 1 H), 1.67 – 1.72 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 154.0, 146.8 (d, $^{1}J_{CF}$ = 240 Hz), 132.5 (d, $^{3}J_{CF}$ = 7.2 Hz), 121.4 (d, $^{3}J_{CF}$ = 6.4 Hz), 116.2 (d, $^{2}J_{CF}$ = 16 Hz), 107.9 (d, $^{2}J_{CF}$ = 17 Hz), 105.5, 79.3, 50.9, 43.7 (br. s), 43.0 (br. s), 28.9 (br. s), 28.6.

Step 3: 4-Fluoro-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure D from tert-butyl 4-(4-fluoro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (100 mg, 0.30 mmol). Yield: quant. LCMS $[M+H]^+$ 236. $^{1}$H-NMR (400 MHz, DMSO-d6) δ ppm 11.52 (s, 1 H), 8.89 (br. s., 1 H), 8.65 (br. s., 1 H), 7.19 (d, $J$ =7.9 Hz, 1 H), 7.02 (td, $J$ =8.1, 5.4 Hz, 1 H), 6.88 - 6.96 (m, 1 H), 4.54 (m, 1 H), 3.04 - 3.17 (m, 2 H), 2.53 - 2.61 (m, 2 H), 1.88 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 4-fluoro-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 87%. LCMS $[M+H]^+$ 481. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.43 (s, 1 H), 8.70 (br. s., 1 H), 8.65 (br. s., 1 H), 7.57 (br. d, $J$ = 8.7 Hz, 2 H), 7.37 (br. d, $J$ = 8.7, 2 H), 7.10 (d, $J$ = 7.9, 1 H), 7.00 (td, $J$ = 8.1, 5.3, 1 H), 6.85 – 6.92 (m, 1 H), 4.54 (tt, $J$ = 12.0, 4.0, 1 H), 3.26 - 3.33 (m, 2 H), 2.90 – 3.00 (m, 2 H), 2.20 – 2.33 (m, 2 H), 1.71 – 1.78 (m, 2H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.0, 146.8 (d, $^{1}J_{CF}$ = 240 Hz), 141.2, 137.6, 132.5 (d, $^{3}J_{CF}$ = 7.5 Hz), 122.2, 121.5 (d, $^{3}J_{CF}$ = 7.0 Hz), 116.2 (d, $^{2}J_{CF}$ = 16 Hz), 107.9 (d, $^{2}J_{CF}$ = 17 Hz), 105.5 (d, $^{4}J_{CF}$ = 2.8 Hz), 85.0, 51.0, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}FIN_4O_2$ 480.0458, found: 480.0562.

*4-(4-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**25**).*

Step 1: tert-butyl 4-(3-chloro-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure A from 1-chloro-3-fluoro-2-nitrobenzene (350 mg, 2.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (400 mg, 2.0 mmol). Yield 93%. LCMS $[M\text{-}isobutene+H]^+$ 300.

Step 2: tert-butyl 4-(4-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure B from tert-butyl 4-(3-chloro-2-nitro-anilino)piperidine-1-carboxylate (660 mg, 1.8 mmol). Yield: 55%. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.41 (s, 1 H), 7.20 (dd, $J$ = 7.8, 0.9 Hz, 1 H), 7.04 (dd, $J$ = 8.1, 0.9 Hz, 1 H), 7.00 (t, $J$ = 8.0 Hz, 1 H), 4.34 (tt, $J$ = 12.2, 4.0 Hz, 1 H), 4.02 – 4.15 (m, 2 H), 2.76 – 2.97 (m, 2 H), 2.14 – 2.23 (m, 2 H), 1.67 – 1.72 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 154.0, 131.0, 126.5, 122.0, 120.9, 113.7, 107.8, 79.3, 50.9, 43.8, 43.1, 28.9, 28.6.

Step 3: 4-chloro-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure C from tert-butyl 4-(4-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (110 mg, 0.31 mmol). Yield: quant. LCMS $[M+H]^+$ 232.

Step 4: The title compound was synthesized according to General procedure A from 4-chloro-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 87%. LCMS $[M+H]^+$ 497. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.40 (br. s, 1 H), 8.69 (s, 1 H), 7.55 – 7.59 (m, 2 H), 7.34 – 7.37 (m, 2 H), 7.22 (dd, *J* = 7.7, 1.0 Hz, 1 H), 7.04 (dd, *J* = 8.1, 1.0 Hz, 1 H), 7.01 (t, *J* = 7.9 Hz, 1 H), 4.40 (tt, *J* = 4.1 Hz, 1 H), 7.26 – 7.31 (m, 2 H), 2.90 – 2.97 (m, 2 H), 2.27 (td, *J* = 12.6, 4.1 Hz, 1 H), 2.25 (td, *J* = 12.6, 4.1 Hz, 1 H), 1.72 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.0, 141.1, 137.3, 131.0, 126.5, 122.2, 122.0, 120.9, 113.7, 107.8, 85.0, 51.0, 43.9, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}ClIN_4O_2$ 496.0163, found: 496.0251.

*4-(4-methoxy-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**27**).*

Step 1: tert-butyl 4-(3-methoxy-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to General procedure A from 1-fluoro-3-methoxy-2-nitrobenzene (170 mg, 1.0 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (200 mg, 1.0 mmol). Yield: 68%. LCMS $[M\text{- isobutene}+H]^+$ 296.

Step 2: tert-butyl 4-(4-methoxy-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate was synthesized according to General procedure F from tert-butyl 4-(3-methoxy-2-nitro-anilino)piperidine-1-carboxylate (230 mg, 0.65 mmol). Yield: 65%. LCMS $[M\text{-isobutene}+H]^+$ 292. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.94 (s, 1 H), 6.94 (t, *J* = 8.1 Hz, 1 H), 6.83 (d, *J* = 8.1 Hz, 1 H), 6.69 (d, *J* = 8.1 Hz, 1 H), 4.31 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.03 – 4.14 (m, 2 H), 3.83 (s, 3 H), 2.76 – 2.96 (m, 2 H), 2.14 – 2.21 8m, 2 H), 1.64 – 1.69 (m, 2 H), 1.44 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 154.1, 144.2, 130.6, 121.4, 117.4, 104.5, 102.6, 79.3, 56.1, 50.5, 43.9 (br. s), 43.1 (br. s), 29.1 (br. s), 28.6.

Step 3: 4-methoxy-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid was synthesized according to General procedure C from tert-butyl 4-(4-

methoxy-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (140 mg, 0.40 mmol). Yield: quant. LCMS $[M+H]^+$ 248. $^1$H-NMR (400 MHz, DMSO-d6) δ ppm 11.03 (s, 1 H), 8.66 (br. s., 1 H), 8.43 (br. s, 1 H), 6.94 - 7.03 (m, 2 H), 6.68 - 6.77 (m, 1 H), 4.50 (br. t, *J* =11.7, 11.7 Hz, 1 H), 3.85 (s, 3 H), 3.45 (br. s., 2 H), 3.03 - 3.17 (m, 2 H), 2.53 - 2.61 (m, 2 H), 1.79 - 1.89 (m, 2 H).

Step 4: The title compound was synthesized according to General procedure A from 4-methoxy-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one;2,2,2-trifluoroacetic acid and 4-iodophenyl isocyanate. Yield: 73%. LCMS $[M+H]^+$ 493. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.94 (br. s, 1 H), 8.69 (s, 1 H), 7.55 – 7.58 (m, 2 H), 7.34 – 7.38 (m, 2 H), 6.94 (t, *J* = 8.1 Hz, 1 H), 6.86 (d, *J* = 7.9 Hz, 1 H), 6.70 (d, *J* = 8.2 Hz, 1 H), 4.37 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.25 – 4.30 (m, 2 H), 3.83 (s, 3 H), 2.90 – 2.97 (m, 2 H), 2.27 (td, *J* = 12.6, 4.1 Hz, 1 H), 2.25 (td, *J* = 12.6, 4.1 Hz, 1 H), 1.68 – 1.74 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.1, 144.2, 141.2, 137.3, 130.7, 122.2, 121.4, 117.4, 104.5, 102.6, 85.0, 56.1, 50.6, 44.0, 29.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}IN_4O_3$ 492.0658, found: 492.0767.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3,4-dichlorophenyl)-N-methylpiperidine-1-carboxamide (**29**).*

The title compound was synthesized according to general procedure H from 7-bromo-3-(4-piperidyl)-1H-benzimidazol-2-one and 3,4-dichloro-N-methyl-aniline. Yield: 43% LCMS $[M+H]^+$ 497. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.28 (br. s, 1 H), 7.59 (d, *J* = 8.8 Hz, 1 H), 7.50 (d, *J* = 2.7 Hz, 1 H), 7.19 (d, *J* = 8.8 Hz, 1 H), 7.59 (d, *J* = 8.7 Hz, 1 H), 7.17 (br. d, *J* = 8.1 Hz, 1 H), 6.97 (t, *J* = 8.1 Hz, 1 H), 4.31 (tt, *J* = 12.3, 4.1 Hz, 1 H), 3.15 (s, 3 H), 2.81 – 2.88 (m, 2 H), 2.12 (td, *J* = 12.5, 4.2 Hz, 1 H), 2.10 (td, *J* = 12.5, 4.2 Hz, 1 H), 1.59 – 1.64 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 159.9, 153.8, 146.8, 131.9, 131.3, 130.6, 128.3, 125.5, 123.9, 123.7, 122.6, 122.3, 108.3, 101.6, 50.5, 45.3, 38.6, 28.5. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{19}BrCl_2N_4O_2$ 496.0068, found: 496.0161.

*4-bromo-1-{1-[2-(4-iodophenyl)acetyl]piperidin-4-yl}-2,3-dihydro-1H-1,3-benzodiazol-2-one (**30**).*

The title compound was synthesized according to general procedure G from 7-bromo-3-(4-piperidyl)-1H-benzimidazol-2-one and 2-(4-iodophenyl)acetic acid. Yield: 70%. LCMS $[M+H]^+$ 540. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 10.82 (br. s, 1 H), 7.68 – 7.72 (m, 2 H), 7.17 (dd, *J* = 8.1, 0.6 Hz, 1 H), 7.11 – 7.14 (m, 2 H), 7.04 (br. d, *J* = 7.9 Hz, 1 H), 6.95 (t, *J* = 8.0 Hz, 1 H), 4.53 – 4.58 (m, 1 H), 4.42 (tt, *J* = 12.3, 4.2 Hz, 1 H), 4.06 – 4.11 (m, 1 H), 3.79 (d, *J* = 15.3 Hz, 1 H), 3. 73 (d, *J* = 15.3 Hz, 1 H), 3.12 – 3.18 (m, 1 H), 2.68 (td, J = 12.9, 2.2 Hz, 1 H), 2.04 (dtd, *J* = 12.6, 12.6, 4.3 Hz, 1 H), 1.98 (dtd, *J* = 12.5, 12.5, 4.3 Hz, 1 H), 1.62 – 1.73 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 168.8, 153.8, 137.5, 136.4, 132.0, 130.5, 128.3, 123.7, 122.3, 108.2, 101.6, 92.7, 50.5, 45.3, 41.3, 29.3, 28.8, 24.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{19}BrIN_3O_2$ 538.9705, found: 538.9825.

*4-chlorophenyl 4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)piperidine-1-carboxylate (**31**).*

In a vial, 7-bromo-3-(4-piperidyl)-1H-benzimidazol-2-one (15 mg, 0.050 mmol) was dissolved in DCM (1.0 mL), then diisopropylethylamine (0.014 mL, 0.10 mmol) and 4-chlorophenyl chloroformate (0.0070 mL, 0.050 mmol) were added. The resulting mixture was stirred at room temperature for 16 h after which it was purified by silica gel chromatography. Yield: 68%. LCMS $[M+H]^+$ 450. 1H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.33 (br. s, 1 H), 7.44 – 7.48 (m, 2 H), 7.35 (br. d, *J* = 7.7 Hz, 1 H), 7.22 – 7.25 (m, 2 H), 7.18 (d, *J* = 8.1 Hz, 1 H), 6.97 (t, *J* = 8.0 Hz, 1 H), 4.45 (tt, *J* = 12.2, 4.0 Hz, 1 H), 4.28 – 4.36 (m, 1 H), 4.15 – 4.22 (m, 1 H), 3.16 – 3.23 (m, 1 H), 3.00 – 3.08 (m, 1 H), 2.27 – 2.42 (m, 2 H), 1.76 – 1.82 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 153.9, 152.9, 150.6, 130.7, 129.7, 129.6, 128.3, 124.3, 123.9, 122.4, 108.4, 101.5, 50.6, 50.0, 44.3, 43.9, 29.0, 28.7. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{17}BrClN_3O_3$ 449.0142, found: 449.0231.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-cyclohexylpiperidine-1-carboxamide (**32**).*

The title compound was synthesized according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and cyclohexyl isocyanate. Yield: 42%. LCMS $[M+H]^+$ 421. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.28 (br. s, 1 H), 7.18 (d, *J* = 7.9 Hz, 1 H), 7.16 (d, *J* = 8.1 Hz, 1 H), 6.95 (T, *J* = 8.0 Hz, 1 H), 6.22 (br. d, *J* = 7.7 Hz, 1 H), 4.28 – 4.34 (m, 1 h), 4.10 – 4.15 (m, 2 H), 3.39 – 3.46 (m, 1 H), 2.72 – 2.78 (m, 2 H), 2.11 – 2.20 (m, 2 H), 1.75 – 1.8 (m, 2 H), 1.63 – 1.73 (m, 4 H), 1.55 – 1.60 (m, 1 H), 1.14 – 1.29 (m, 4 H), 1.06 – 1.12 (m, 1 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 157.1, 153.9, 130.8, 128.3, 123.7, 122.4, 108.1, 101.5, 51.2, 49.7, 43.7, 33.6, 28.9, 25.9, 25.6, 19.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{25}BrN_4O_2$ 420.1161, found: 420.1290.

*4-bromo-1-[1-(4-iodobenzoyl)piperidin-4-yl]-2,3-dihydro-1H-1,3-benzodiazol-2-one (**33**).*

In a vial, 7-bromo-3-(4-piperidyl)-1H-benzimidazol-2-one (15 mg, 0.050 mmol) was dissolved in THF (1.0 mL), then diisopropylethylamine (0.014 mL, 0.10 mmol) and 4-iodobenzoyl chloride (14 mg, 0.050 mmol) were added. The resulting mixture was stirred at room temperature for 16 h after which it was concentrated and purified by silica gel chromatography. Yield: 88%. LCMS $[M+H]^+$ 526. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.24 (br. s, 1 H), 7.75 – 7.79 (m, 2 H), 7.31 (br. d, 7.8 Hz, 1 H), 7.19 – 7.22 (m, 2 H), 7.10 (br. d, 7.9 Hz, 1 H), 6.89 (t, 8.0 Hz, 1 H), 4.50 – 4.62 (m, 1 H), 4.39 (tt, *J* = 12.2, 4.0 Hz, 1 H), 3.54 – 3.65 (m, 1 H), 3.09 – 3.19 (m, 1 H), 2.77 – 2.89 (m, 1 H), 2.13 – 2.29 (m, 2 H), 1.56 – 1.79 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 168.8, 153.9, 137.7, 136.1, 130.6, 129.4, 128.2, 123.8, 122.4, 108.5, 101.5, 96.7, 50.7, 47.1, 29.4, 28.7. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{17}BrIN_3O_2$ 524.9549, found: 524.9664.

*4-bromo-1-[1-(4-iodobenzenesulfonyl)piperidin-4-yl]-2,3-dihydro-1H-1,3-benzodiazol-2-one (**34**).*

In a vial, 7-bromo-3-(4-piperidyl)-1H-benzimidazol-2-one (15 mg, 0.050 mmol) was dissolved in pyridine (1.0 mL), then diisopropylethylamine (0.014 mL, 0.10 mmol) and 4-iodobenzenesulfonyl chloride (15 mg, 0.050 mmol) were added. The resulting mixture was stirred at 70 °C for 16 h after which it was diluted with MeOH (2 mL) and purified by preparative HPLC. Yield: 25%. LCMS $[M+H]^+$ 563. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.28 (br. s, 1 H), 8.07 – 8.10 (m, 2 H), 7.56 – 7.59 (m, 2 H), 7.15 (dd, *J* = 8.1, 0.5 Hz, 1 H), 7.01 (bd, *J* = 7.9 Hz, 1 H), 6.92 (t, *J* =8.0 Hz, 1 H), 4.21 (tt, *J* = 12.2, 4.1 Hz, 1 H), 3.78 – 3.83 (m, 2 H), 2.51 – 2.57 (m, 2 H), 2.27 – 2.35 (m, 2 H), 1.71 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 153.8, 138.9, 136.3, 130.8, 129.6, 128.3, 123.8, 122.3, 107.9, 102.0, 101.5, 49.8, 46.0, 28.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{18}H_{17}BrIN_3O_3S$ 560.9219, found: 560.9319.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(6-methoxy-5-methylpyridin-3-yl)piperidine-1-carboxamide (**35**).*

The title compound was synthesized according to general procedure H from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 3-amino-6-methoxy-5-methylpyridine. Yield: 56%. LCMS $[M+H]^+$ 460. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.50 (s, 1 H), 8.03 (d, *J* = 2.5 Hz, 1H), 7.65 (dd, *J* = 2.5, 0.7 Hz, 1 H), 7.26 (d, *J* = 7.8 Hz, 1 H), 7.17 (dd, *J* = 8.1, 0.4 Hz, 1 H), 6.96 (t, *J* = 8.1 Hz, 1 H), 4.39 (tt, 12.2, 4.1 Hz, 1 H), 4.26 – 4.31 (m, 2 H), 3.84 (s, 3 H), 2.90 – 2.96 (m, 2 H), 2.22 – 2.31 (m, 2 H), 2.13

(s, 3 H), 1.73 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 157.7, 155.4, 153.9, 135.8, 135.7, 133.1, 133.0, 131.6, 130.8, 128.3, 123.7, 122.4, 119.3, 108.2, 101.5, 53.5, 51.1, 43.8, 29.0, 16.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{22}BrN_5O_3$ 459.0906, found: 459.1041.

*3-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**36**).*

Step 1: tert-butyl 3-(4-chloro-2-nitro-anilino)piperidine-1-carboxylate was synthesized according to general procedure B from 4-chloro-1-fluoro-2-nitro-benzene (88 mg, 0.50 mmol) and tert-butyl 4-aminopiperidine-1-carboxylate (100 mg, 0.50 mmol) in 95% yield. LCMS $[M\text{-isobutene}+H]^+$ 300.

Step 2: tert-butyl 3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate was synthesized from tert-butyl 3-(4-chloro-2-nitro-anilino)piperidine-1-carboxylate (100 mg, 0.28 mmol) according to general procedure C. Yield: 67%. LCMS $[M+H]^+$ 326. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.1 (s, 1 H), 7.36 (d, *J* = 8.4 Hz, 1 H), 7.02 (dd, *J* = 8.4, 2.0 Hz, 1 H), 7.01 (d, *J* = 2.0 Hz, 1 H), 4.12 (tt, *J* = 11.8, 4.3 Hz, 1 H), 3.86 – 3.99 (m, 2 H), 3.30 – 3.48 (m, 1 H), 2.70 – 2.90 (m, 1 H), 2.23 – 2.32 (m, 1 H), 1.76 – 1.84 (m, 2 H), 1.46 – 1.55 (m, 1 H), 1.41 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.4, 154.1, 130.1, 128.5, 125.5, 120.5, 110.5, 109.2, 79.5, 49.7 (br. s), 46.2 (br. s), 45.1 (br. s), 44.0 (br. s), 43.1 (br. s), 28.5, 27.5, 25.0 (br. s).

Step 3: 6-chloro-3-(3-piperidyl)-1H-benzimidazol-2-one; trifluoroacetic acid was synthesized from tert-butyl 3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)piperidine-1-carboxylate (63 mg, 0.18 mmol) according to general procedure D. Yield: quant. LCMS $[M+H]^+$ 232.

Step 4: The title compound was synthesized from 6-chloro-3-(3-piperidyl)-1H-benzimidazol-2-one; trifluoroacetic acid according to general procedure A. Yield: 86%. LCMS $[M+H]^+$ 496. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.12 (s, 1 H), 8.66 (s, 1 H), 7.53 – 7.56 (m, 2 H), 7.39 (d, *J* = 8.4 Hz, 1 H), 7.31 – 7.35 (m, 2 H), 7.04 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.02 (d, *J* = 2.1 Hz, 1 H), 4.12 – 4.22 (m, 3 H), 3.48 (br. t, *J* = 11.7 Hz, 1 H), 2.85 – 2.91 (m, 1 H), 2.27 – 2.35 (m, 1 H), 1.79 – 1.88 (m, 2 H), 1.54 – 1.64 (m, 1 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.0, 154.2, 141.0, 137.3, 130.0, 128.7, 125.5, 122.3, 120.5, 110.5, 109.2, 85.2, 49.9, 46.1, 44.1, 27.7, 25.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}ClIN_4O_2$ 496.0163, found: 496.0256.

*3-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)pyrrolidine-1-carboxamide (**37**).*

Step 1: tert-butyl 3-(4-chloro-2-nitro-anilino)pyrrolidine-1-carboxylate was synthesized according to general procedure B from 4-chloro-1-fluoro-2-nitro-benzene (180 mg, 1.0 mmol) and tert-butyl tert-butyl 3-aminopyrrolidine-1-carboxylate (190 mg, 1.0 mmol) in 82% yield. LCMS $[M\text{-isobutene}+H]^+$ 286.

Step 2: tert-butyl 3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)pyrrolidine-1-carboxylate was synthesized from tert-butyl 3-(4-chloro-2-nitro-anilino)pyrrolidine-1-carboxylate (280 mg, 0.82 mmol) according to general procedure C. Yield 30%. LCMS $[M\text{-isobutene}+H]^+$ 282. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.11 (s, 1 H), 7.16 – 7.20 (m, 1 H), 7.04 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.01 (d, *J* = 2.1 Hz, 1 H), 4.92 – 5.00 (m, 1 H), 3.52 – 3.66 (m, 3 H), 2.43 – 2.50 (m, 1 H), 2.09 – 2.15 (m, 1 H), 1.41 (app. br. d, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.1, 153.9, 130.0, 128.6, 125.6, 120.6, 109.9, 109.3, 79.0, 51.4, 50.8, 46.7, 46.6, 45.0, 44.7, 28.6, 28.4, 27.4.

Step 3: 6-chloro-3-pyrrolidin-3-yl-1H-benzimidazol-2-one; trifluoroacetic acid was synthesized from tert-butyl 3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)pyrrolidine-1-carboxylate (82 mg, 0.24 mmol) according to general procedure D. Yield: quant. LCMS $[M+H]^+$ 238.

Step 4: The title compound was synthesized from 6-chloro-3-pyrrolidin-3-yl-1H-benzimidazol-2-one; trifluoroacetic acid according to general procedure A. Yield 77%. LCMS $[M+H]^+$ 482. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.15 (s, 1 H), 8.35 (s, 1 H), 7.54 – 7.57 (m, 2 H), 7.38 – 7.41 (m, 2 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 7.06 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.03 (d, *J* = 2.1 Hz, 1 H), 5.05 (p, *J* = 8.3 Hz, 1 H), 3.80 (dd, *J* = 10.3, 7.9 Hz, 1 H), 3.70 – 3.76 (m, 2 H), 3.43 – 3.49 (m, 1 H), 2.52 – 2.57 (m, 1 H), 2.16 – 2.22 (m, 1 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.1, 140.9, 137.3, 130.0, 128.5, 125.7, 122.0, 121.9, 120.7, 110.1, 109.3, 85.0, 51.2, 46.9, 45.1, 27.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{18}H_{16}ClIN_4O_2$ 482.0006, found: 482.0113.

*3-[4-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)cyclohexyl]-1-(4-iodophenyl)urea (**38**).*

Step 1: tert-butyl N-[4-(4-chloro-2-nitro-anilino)cyclohexyl]carbamate was synthesized according to general procedure B from 4-chloro-1-fluoro-2-nitro-benzene (180 mg, 1.0 mmol) and tert-butyl tert-butyl N-(4-aminocyclohexyl)carbamate (210 mg, 1.0 mmol). Yield: 32%. LCMS $[M\text{-isobutene}+H]^+$ 314.

Step 2: tert-butyl N-[4-(5-chloro-2-oxo-3H-benzimidazol-1-yl)cyclohexyl]carbamate (85:15 mixture of isomers) was synthesized from tert-butyl N-[4-(2-amino-4-chloro-anilino)cyclohexyl]carbamate (100 mg, 0.27 mmol) according to general procedure C. Yield: 80%. LCMS $[M\text{-}Boc+H]^+$ 266. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.00 (br. s, 1 H), 7.50 (br. d, *J* = 8.5 Hz, 0.85 H), 7.35 (br. d, *J* = 8.2 Hz, 0.15 H), 7.20 – 7.30 (m, 1 H), 7.03 (dd, *J* = 8.5, 2.1 Hz, 0.85 H), 6.97 – 7.00 (m, 1 H), 6.76 – 6.80 (m, 0.15 H), 4.06 – 4.17 (m, 1 H), 3.66 – 3.72 (m, 0.85 H), 3.37 – 3.45 (m, 0.15 H), 2.26 – 2.34 (m, 1.7 H), 2.14 – 2.23 (m, 0.3 H), 1.87 – 1.92 (m, 0.3 H), 1.79 – 1.86 (m, 1.7 H), 1.60 – 1.69 (m, 2 H), 1.38 – 1.49 (m, 11 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.7, 155.3, 154.1, 130.0, 128.5, 125.2, 120.4, 120.2, 111.0, 110.7, 109.0, 108.6, 78.1, 77.9, 51.9, 51.5, 48.5, 44.3, 32.2, 30.1, 28.8, 28.7, 28.3, 24.8.

Step 3: 3-(4-aminocyclohexyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid was synthesized from tert-butyl N-[4-(5-chloro-2-oxo-3H-benzimidazol-1-yl)cyclohexyl]carbamate (79 mg, 0.21 mmol) according to general procedure D. Yield: quant. LCMS $[M+H]^+$ 266.

Step 4: The title compound was synthesized from 3-(4-aminocyclohexyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid according to general procedure A. Yield: 82%. LCMS $[M+H]^+$ 510. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.04 (br. s, 1 H), 8.57 (s, 1 H), 7.53 – 7.57 (m, 2 H), 7.31 (d, 8.4 Hz, 1 H), 7.26 – 7.29 (m, 2 H), 7.06 (dd, *J* = 8.4, 2.1 Hz, 1 H), 7.00 (d, *J* = 2.1 Hz, 1 H), 6.59 (br. d, *J* = 6.9 Hz, 1 H), 4.17 (tt, *J* = 12.4, 3.7 Hz, 1 H), 3.86 – 3.90 (m, 1 H), 2.19 – 2.28 (m, 2 H), 1.84 – 1.90 (m, 2 H), 1.67 – 1.74 (m, 2 H), 1.56 – 1.62 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.8, 154.1, 140.8, 137.7, 130.1, 128.7, 125.3, 120.4, 120.3, 110.3, 109.2, 83.9, 51.8, 43.2, 30.0, 24.7. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{20}ClIN_4O_2$ 510.0319, found: 510.0401.

*3-[3-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)propyl]-1-(4-iodophenyl)urea* ***(39)****.*

Step 1: tert-butyl N-[3-(4-chloro-2-nitro-anilino)propyl]carbamate was synthesized according to general procedure B from 4-chloro-1-fluoro-2-nitro-benzene (180 mg, 1.0 mmol) and tert-butyl N-(3-aminopropyl)carbamate (170 mg, 1.0 mmol). Yield: 95%. LCMS $[M\text{-}Boc+H]^+$ 274.

Step 2: tert-butyl N-[3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)propyl]carbamate was synthesized according to general procedure C from tert-butyl N-[3-(4-chloro-2-nitro-anilino)propyl]carbamate 300 mg, 0.91 mmol). Yield: 80%. LCMS $[M\text{-}Boc+H]^+$ 270. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.02 (s, 1 H), 7.15 (d, *J* = 8.3 Hz, 1 H), 7.04 (dd, *J* =

8.3, 2.0 Hz, 1 H), 7.00 (d, *J* = 2.0 Hz, 1 H), 6.84 (br. t, *J* = 5.3 Hz, 1 H), 3.77 (br. t, *J* = 7.1 Hz, 2 H), 2.94 (br. q, *J* = 6.5 Hz, 2 H), 1.73 (br. quint., *J* = 7.0 Hz, 2 H), 1.37 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 156.0, 154.6, 129.9, 129.6, 125.4, 120.6, 109.3, 109.1, 78.0, 38.3, 38.0, 28.7, 28.5.

Step 3: 3-(3-aminopropyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid was synthesized according to general procedure D from tert-butyl N-[3-(5-chloro-2-oxo-3H-benzimidazol-1-yl)propyl]carbamate (270 mg, 0.73 mmol). Yield: quant. LCMS $[M+H]^+$ 232.

Step 4: The title compound was synthesized from 3-(3-aminopropyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid according to general procedure A. Yield: 83%. LCMS $[M+H]^+$ 470. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.05 (br. s, 1 H), 8.69 (s, 1 H), 7.50 – 7.54 (m, 2 H), 7.23 – 7.26 (m, 2 H), 7.16 (d, *J* = 8.3 Hz, 1 H), 7.05 (dd, *J* = 8.3, 2.0 Hz, 1 H), 7.01 (d, *J* = 2.0 Hz, 1 H), 6.26 (br. t, 5.7 Hz, 1 H), 3.82 (br. t, *J* = 6.9 Hz, 2 H), 3.08 (br. q, *J* = 6.4 Hz, 2 H), 1.77 (br. p, *J* = 6.8 Hz, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.4, 154.7, 140.9, 137.6, 129.9, 129.6, 125.4, 120.7, 120.4, 109.3, 109.2, 83.9, 38.2, 37.0, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{17}H_{16}ClIN_4O_2$ 470.0006, found: 470.0105.

*3-[2-(5-chloro-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)ethyl]-1-(4-iodophenyl)urea (**40**).*

Step 1: tert-butyl N-[2-(4-chloro-2-nitro-anilino)ethyl]carbamate was synthesized according to general procedure B from 4-chloro-1-fluoro-2-nitro-benzene (180 mg, 1.0 mmol) and tert-butyl N-(2-aminoethyl)carbamate (160 mg, 1.0 mmol). Yield: 90%. LCMS $[M\text{-}Boc+H]^+$ 260.

Step 2: tert-butyl N-[2-(5-chloro-2-oxo-3H-benzimidazol-1-yl)ethyl]carbamate was synthesized according to general procedure C from tert-butyl N-[2-(4-chloro-2-nitro-anilino)ethyl]carbamate (280 mg, 0.90 mmol). Yield: 77%. LCMS $[M\text{-}Boc +H]^+$ 212. $^{1}$H-NMR (400 MHz, DMSO-d6): δ (ppm)10.97 (s, 1 H), 7.04 – 7.07 (m, 1 H), 7.02 – 7.04 (m, 1 H), 6.96 – 6.99 (m, 1 H), 6.89 (br. t, *J* = 5.9 Hz, 1 H), 3.78 (br. t, *J* = 5.9 Hz, 2 H), 3.17 (br. q, *J* = 5.9 Hz, 2 H), 1.18 – 1.29 (m, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 156.1, 154.6, 130.3, 129.9, 125.2, 120.4, 109.0, 108.9, 78.1, 38.9, 28.5, 28.2.

Step 3: 3-(2-aminoethyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid was synthesized according to general procedure D from tert-butyl N-[2-(5-chloro-2-oxo-3H-benzimidazol-1-yl)ethyl]carbamate (215 mg, 0.69 mmol). Yield: quant. LCMS $[M+H]^+$ 212.

Step 4: The title compound was synthesized from 3-(2-aminoethyl)-6-chloro-1H-benzimidazol-2-one; trifluoroacetic acid according to general procedure A. Yield: 75%. LCMS

$[M+H]^+$ 456. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.02 (s, 1 H), 8.65 (s, 1 H), 7.50 – 7.53 (m, 2 H), 7.19 – 7.22 (m, 2 H), 7.14 (d, *J* = 8.3 Hz, 1 H), 7.03 (dd, *J* = 8.3, 2.0 Hz, 1 H), 6.99 (d, *J* = 2.0 Hz, 1 H), 6.27 (br. t, *J* = 5.9 Hz, 1 H), 3.87 (br. t, *J* = 6.2 Hz, 2 H), 3.31 – 3.36 (multiplet overlapping with solvent, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.6, 154.7, 140.7, 137.6, 130.0, 129.9, 125.3, 120.6, 120.5, 109.2, 109.1, 84.2, 40.6, 38.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{16}H_{14}ClIN_4O_2$ 455.9850, found: 455.9935.

*4-(4-bromo-1H-1,3-benzodiazol-1-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**41**).*

Step 1: 4-bromo-1-(4-piperidinyl)-1H-benzimidazole. A vial was charged with tert-butyl 4-(2-amino-3-bromo-anilino)piperidine-1-carboxylate (37 mg, 0.10 mmol) and formic acid (1 mL). the reaction mixture was stirred at reflux for 16 h after which it was concentrated under reduced pressure. The crude material was used in the next step without further purification. LCMS $[M+H]^+$ 281.

Step 2: The title compound was synthesized from 4-bromo-1-(4-piperidinyl)-1H-benzimidazole; formic acid according to general procedure A. Yield 53%. LCMS $[M+H]^+$ 525. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 8.71 (s, 1 H), 8.53 (s, 1 H), 7.76 (d, *J* = 7.9 Hz, 1 H), 7.55 – 7.59 (m, 2 H), 7.46 (d, *J* = 7.9 Hz, 1 H), 7.35 – 7.38 (m, 2 H), 7.22 (t, *J* = 7.9 Hz, 1 H), 4.68 (tt, *J* = 11.8, 4.0 Hz, 1 H), 4.31 – 4.36 (m, 2 H), 3.00 – 3.06 (m, 2 H), 2.06 – 2.11 (m, 2 H), 1.96 – 2.05 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.8, 142.9, 141.9, 141.1, 137.3, 134.5, 125.0, 124.0, 122.3, 112.9, 110.9, 85.1, 53.5, 43.6, 32.2. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}BrIN_4O$ 523.9709, found: 523.9841.

*4-(7-bromo-2-oxo-2,3-dihydro-1,3-benzoxazol-3-yl)-N-(4-iodophenyl)piperidine-1-carboxamide (**42**).*

Step 1: tert-butyl 4-(3-bromo-2-hydroxy-anilino)piperidine-1-carboxylate. A mixture of 2-amino-6-bromo-phenol (190 mg, 1.0 mmol), tert-butyl 4-oxopiperidine-1-carboxylate (200 mg, 1.0 mmol), and $NaBH(OAc)_3$ (1200mg, 5.5 mmol) was stirred in DCM (10 mL) at 20 °C for 3 h. The mixture was then filtered through celite and purified by silica gel chromatography followed by recrystallization from DCM. Yield 135 mg (36%), a second crop afforded an additional 160 mg (43%). LCMS $[M\text{-isobutene}+H]^+$ 315. $^1$H-NMR (400 MHz, DMSO-d6): δ ppm 8.74 (br. s, 1 H), 6.69 (dd, *J* = 7.9, 1.5 Hz, 1 H), 6.64 (t, *J* = 7.9 Hz, 1 H), 6.60 (dd, *J* = 7.9, 1.5 Hz, 1 H), 4.66 – 4.79 (m, 1 H), 3.84 – 3.94 (m, 2 H), 3.39 – 3.46 (m, 1 H), 2.79 – 2.97 (m, 2 H), 1.85 – 1.91 (m, 2 H), 1.41 (s, 9 H), 1.24 – 1.32 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.4, 140.8, 139.3, 122.4, 119.4, 111.7, 110.2, 79.1, 49.5, 43.2 (br. s), 32.0, 28.6.

Step 2: A vial was charged with tert-butyl 4-(3-bromo-2-hydroxy-anilino)piperidine-1-carboxylate (160 mg, 0.43 mmol), N-ethyl-N-isopropyl-propan-2-amine (0.15 mL, 0.86 mmol), and DCM (5 mL), then trichloromethyl carbonochloridate (0.026 mL, 0.21 mmol) in DCM (1 mL) was added. The resulting mixture was stirred at 20 °C for 10 min after which it was purified by silica gel chromatography which afforded 4-(7-bromo-2-oxo-1,3-benzoxazol-3-yl)-N-tert-butyl-piperidine-1-carboxamide (150 mg, 84%). LCMS $[M\text{-isobutene}+H]^+$ 341.

Step 3: 7-bromo-3-(4-piperidyl)-1,3-benzoxazol-2-one; trifluoroacetic acid was synthesized according to general procedure D from 4-(7-bromo-2-oxo-1,3-benzoxazol-3-yl)-N-tert-butyl-piperidine-1-carboxamide (150 mg, 0.38 mmol). Yield: quant. LCMS $[M+H]^+$ 297.

Step 4: The title compound was synthesized from 7-bromo-3-(4-piperidyl)-1,3-benzoxazol-2-one; trifluoroacetic acid according to general procedure A. Yield 80%. LCMS $[M+H]^+$ 542. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 8.69 (s, 1 H), 7.55 – 7.58 (m, 2 H), 7.43 (dd, *J* = 8.0, 0.8 Hz, 1 H), 7.33 – 7.37 (m, 3 H), 7.18 (t, *J* = 8.0 Hz, 1 H), 4.36 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.26 – 4.32 (m, 2 H), 2.91 – 2.98 (m, 2 H), 2.11 – 2.19 (m, 2 H), 1.86 – 1.91 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 152.4, 141.1, 140.3, 137.4, 131.8, 125.7, 125.2, 122.2, 109.7, 101.7, 85.1, 52.7, 43.6, 28.6. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{17}BrIN_3O_3$ 540.9498, found: 540.9590.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-chlorophenyl)piperidine-1-carboxamide (**43**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-chlorophenyl isocyanate in 74% yield. LCMS $[M+H]^+$ 449. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.71 (s, 1 H), 7.52 – 7.55 (m, 2 H), 7.27 – 7.30 (m, 2 H), 7.26 (dd, *J* = 8.0, 0.5 Hz, 1 H), 7.17 (dd, *J* = 8.0, 0.5 Hz, 1 H), 6.96 (t, *J* = 8.0, 1 H), 4.40 (tt, 12.2, 4.1 Hz, 1 H), 4.28 – 4.32 (m, 2 H), 2.91 – 2.97 (m, 2 H), 2.23 – 2.31 (m, 2 H), 1.73 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.0, 153.9, 140.2, 130.8, 128.6, 128.3, 125.6, 123.7, 122.4, 121.4, 108.1, 101.5, 51.0, 43.9, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}BrClN_4O_2$ 448.0302, found: 448.0402.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-bromophenyl)piperidine-1-carboxamide (**44**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-bromophenyl isocyanate. Yield 80%. LCMS $[M+H]^+$ 493. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.31 (br. s, 1 H), 8.72 (s, 1 H), 7.49 (d, J = 9.0 Hz, 2 H), 7.42 (d, J = 9.0 Hz, 2 H), 7.26 (d, *J* = 7.9 Hz, 1 H), 7.17 (dd, *J* = 8.1, 0.5 Hz, 1 H), 6.96 (t, *J* = 8.0, 1 H), 4.41 (tt, 12.1, 4.0 Hz, 1 H), 4.26 – 4.34 (m, 2 H), 2.90 – 3.00 (m, 2 H), 2.21 – 2.33 (m, 2 H), 1.72 – 1.79 (m, 2 H). HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}Br_2N_4O_2$ 491.9797, found: 491.9883.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-methylphenyl)piperidine-1-carboxamide (**45**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-tolyl isocyanate. Yield 77%. LCMS $[M+H]^+$ 429. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.48 (s, 1 H), 7.35 – 7.38 (m, 2 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 7.17 (dd, , *J* = 8.0, 0.5 Hz, 1 H), 7.03 – 7.06 (m, 2 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 4.39 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.27 – 4.31 (m, 2 H), 2.88 – 2.95 (m, 2 H), 2.22 – 2.30 (m, 2 H), 2.23 (s, 3 H), 1.71 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.3, 153.9, 138.5, 130.9, 130.8, 129.2, 128.3, 123.7, 122.4, 120.2, 108.1, 101.5, 51.1, 43.9, 29.0, 20.8. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}BrN_4O_2$ 428.0848, found: 428.0965.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-tert-butylphenyl)piperidine-1-carboxamide (**46**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-*tert*butylphenyl isocyanate. Yield 65%. LCMS $[M+H]^+$ 471. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.23 (s, 1 H), 8.43 (s, 1 H), 7.30 – 7.33 (m, 2 H), 7.16 – 7.20 (m, 3 H), 7.09 (dd, *J* = 8.1, 0.5 Hz, 1 H), 6.89 (t, *J* = 8.1, 1 H), 4.32 (tt, 12.2, 4.1 Hz, 1 H), 4.19 – 4.24 (m, 2 H), 2.82 – 2.88 (m, 2 H), 2.16 – 2.24 (m, 2 H), 1.65 – 1.70 (m, 2 H), 1.90 (s, 9 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.4, 153.9, 144.4, 138.5, 130.8, 128.3, 125.3, 123.7, 122.4, 119.9, 108.1, 101.5, 51.1, 43.9, 34.3, 31.8, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{23}H_{27}BrN_4O_2$ 470.1317, found: 470.1435.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-methoxyphenyl)piperidine-1-carboxamide (**47**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-methoxyphenyl isocyanate in 54% yield. LCMS $[M+H]^+$ 445. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (s, 1 H), 8.42 (s, 1 H), 7.35 – 7.39 (m, 2 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 7.16 (d, *J* = 8.0, 0.5 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 6.82 – 6.85 (m, 2 H), 4.39 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.26 – 4.31 (m, 2 H), 3.71 (s, 3 H), 2.88 – 2.94 (m, 2 H), 2.22 – 2.31 (m, 2 H), 1.71 – 1.76 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.5, 154.9, 153.9, 134.1, 130.8, 128.3, 123.7, 122.4, 122.0, 114.0, 108.1, 101.5, 55.6, 51.1, 43.9, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{21}BrN_4O_3$ 444.0797, found: 444.0908.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-nitrophenyl)piperidine-1-carboxamide (**48**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-nitrophenyl isocyanate. Yield 90%. LCMS $[M+H]^+$ 460. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.24 (s, 1 H), 9.23 (s, 1 H), 8.08 – 8.11 (m, 2 H), 7.67 – 7.70 (m, 2 H), 7.21 (d, *J* = 8.0 Hz, 1 H), 7.10 (d, *J* = 8.0 Hz, 1 H), 6.89 (t, *J* = 8.0 Hz, 1 H), 4.36 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.23 – 4.28 (m, 2 H), 2.90 – 2.96 (m, 2 H), 2.19 – 2.27 (m, 2 H), 1.68 – 1.73 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.3, 153.9, 148.1, 141.3, 130.8, 128.3, 125.2, 123.7, 122.4, 118.8, 108.2, 101.5, 50.9, 44.0, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{18}BrN_5O_4$ 459.0542, found: 459.0626.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-[4-(trifluoromethyl)phenyl]piperidine-1-carboxamide (**49**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-(trifluoromethyl)phenyl isocyanate. Yield 63%. LCMS $[M+H]^+$ 483. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (s, 1 H), 8.98 (s, 1 H), 7.71 – 7.74 (m, 2 H), 7.58 – 7.62 (m, 2 H), 7.27 (d, *J* = 8.0 Hz, 1 H), 7.17 (dd, *J* = 8.0 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 4.42 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.29 – 4.34 (m, 2 H), 2.94 – 3.01 (m, 2 H), 2.25 – 2.33 (m, 2 H),1.75 – 1.80 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.7, 153.9, 145.0, 130.8, 128.3, 126.1 (br. s), 125.1 (q, *J* = 272 Hz), 123.7, 122.4, 121.9 (q, *J* = 32.7 Hz), 119.3, 108.2, 101.5, 51.0, 43.9, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{18}BrF_3N_4O_2$ 482.0565, found: 482.0670.

***E**thyl-4-{[4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-piperidine-1-carbonyl]-amino}-benzoate (**50**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and ethyl 4-isocyanatobenzoate in 76% yield. LCMS $[M+H]^+$ 487. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.98 (s, 1 H), 7.84 – 7.87 (m, 2 H), 7.64 – 7.67 (m, 2 H), 7.27 (d, *J* = 8.0 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 4.41 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.30 – 4.34 (m, 2 H), 4.28 (q, *J* = 7.1 Hz, 2 H), 2.94 – 3.00 (m, 2 H), 2.24 – 2.32 (m, 2 H), 1.74 – 1.79 (m, 2 H), 1.31 (t, *J* = 7.1 Hz, 3 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 166.0, 154.6, 153.9, 145.9, 130.8, 130.4, 128.3, 123.7, 122.9, 122.4, 118.8, 108.2, 101.5, 60.7, 51.0, 44.0, 29.1, 14.7. HRMS (EI): m/z $[M]^+$ Calcd for $C_{22}H_{23}BrN_4O_4$ 486.0903, found: 486.1007.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-acetamidophenyl)piperidine-1-carboxamide (**51**).*

The title compound was synthesized from according to general procedure H from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-aminoacetanilide in 45% yield. LCMS $[M+H]^+$ 472. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 9.78 (s, 1 H), 8.51 (s, 1 H), 7.42 – 7.45 (m, 2 H), 7.36 – 7.40 (m, 2 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 4.39 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.26 – 4.31 (m, 2 H), 2.89 – 2.95 (m, 2 H), 2.22 – 2.30 (m, 2 H), 2.01 (s, 3 H), 1.71 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 168.2, 155.3, 153.9, 136.3, 134.1, 130.8, 128.3, 123.7, 122.4, 120.6, 119.7, 108.1, 101.5, 51.1, 43.9, 29.0, 24.3. HRMS (EI): m/z $[M]^+$ Calcd for $C_{21}H_{22}BrN_5O_3$ 471.0906, found: 471.1004.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-[4-(dimethylamino)phenyl]piperidine-1-carboxamide (**52**).*

The title compound was synthesized from according to general procedure H from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and N,N-dimethyl-p-phenylenediamine. Yield 25%. LCMS $[M+H]^+$ 458. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.28 (s, 1 H), 7.26 – 7.28 (m, 2 H), 7.24 (d, *J* = 8.0 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 1 H), 6.96 (d, *J* = 8.0 Hz, 1 H), 6.65 – 6.68 (m, 2 H), 4.38 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.24 – 4.30 (m, 2 H), 2.86 – 2.92 (m, 2 H), 2.82 (s, 6 H), 2.22 – 2.30 (m, 2 H), 1.70 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.7, 153.9, 146.9, 130.9, 130.8, 128.3, 123.7, 122.4, 122.2, 113.2, 108.1, 101.5, 51.2, 43.9, 41.2, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{21}H_{24}BrN_5O_2$ 457.1113, found: 457.1219.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3,4-dichlorophenyl)piperidine-1-carboxamide (**53**).*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 3,4-dichlorophenyl isocyanate. Yield 88%.

LCMS $[M+H]^+$ 483. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.24 (s, 1 H), 8.79 (s, 1 H), 7.81 (t, *J* = 1.3 Hz, 1 H), 7.42 (d, *J* = 1.3 Hz, 1 H), 7.205 (d, *J* = 7.9 Hz, 1 H), 7.10 (dd, *J* = 8.1, 0.5 Hz, 1 H), 6.88 (t, *J* = 8.0 Hz, 1 H), 7.25 (tt, *J* = 12.1, 4.0 Hz, 1 H), 4.18 – 4.26 (m, 2H), 2.84 – 2.94 (m, 2 H), 2.14 – 2.26 (m, 2 H), 1.65 – 1.72 (m, 2 H). HRMS (EI): m/z $[M]^+$ Calcd for $C_{19}H_{17}BrCl_2N_4O_2$ 481.9912, found: 482.0002.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(4-chloro-3-methoxyphenyl)piperidine-1-carboxamide (**54**).*

The title compound was synthesized from according to general procedure H from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 4-chloro-3-methoxyaniline. Yield 54%. LCMS $[M+H]^+$ 479. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (br. s, 1 H), 8.70 (s, 1 H), 7.42 (d, *J* = 2.2 Hz, 1 H), 7.26 (d, *J* = 8.0 Hz, 1 H), 7.25 (d, *J* = 8.6 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 1 H), 7.15 (dd, *J* = 8.6, 2.2 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 4.40 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.27 – 4.33 (m, 2 H), 3.81 (s, 3 H), 2.92 – 2.98 (m, 2 H), 2.23 – 2.32 (m, 2 H), 1.74 – 1.78 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 154.9, 154.7, 153.9, 141.5, 130.8, 129.7, 128.3, 123.7, 122.4, 113.6, 112.4, 108.1, 104.5, 101.5, 56.2, 51.1, 43.9, 29.0. HRMS (EI): m/z $[M]^+$ Calcd for $C_{20}H_{20}BrClN_4O_3$ 478.0407, found: 478.0503.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3-methoxy-4-methylphenyl)piperidine-1-carboxamide (**55**).*

The title compound was synthesized from according to general procedure H from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 3-methoxy-4-methylaniline. Yield 60%. LCMS $[M+H]^+$ 459. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (s, 1 H), 8.49 (s, 1 H), 7.26 (d, *J* = 8.0 Hz, 1 H), 7.19 (d, *J* = 1.9 Hz, 1 H), 7.17 (dd, *J* = 8.0, 0.5 Hz, 1 H), 7.00 (dd, *J* = 8.0, 1.9 Hz, 1 H), 6.94 – 6.98 (m, 2 H), 4.39 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.27 – 4.32 (m, 2 H), 3.74 (s, 3 H), 2.89 – 2.96 (m, 2 H), 2.23 – 2.31 (m, 2 H), 2.07 (s, 3 H), 1.73 – 1.75 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 157.5, 155.2, 153.9, 140.2, 130.8, 130.2, 128.3, 123.7, 122.4, 118.9, 111.6, 108.1, 103.0, 101.5, 55.4, 51.1, 43.9, 29.1, 15.9. HRMS (EI): m/z $[M]^+$ Calcd for $C_{21}H_{23}BrN_4O_3$ 458.0954, found: 458.1059.

*4-(4-bromo-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3,4-dimethoxyphenyl)piperidine-1-carboxamide **(56)**.*

The title compound was synthesized from according to general procedure A from 4-bromo-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one and 3,4-dimethoxyphenyl isocyanate. Yield 58%. LCMS $[M+H]^+$ 475. $^1$H-NMR (400 MHz, DMSO-d6): δ (ppm) 11.30 (s, 1 H), 8.41 (s, 1 H), 7.25 (d, *J* = 8.0 Hz, 1 H), 7.19 (d, *J* = 2.4 Hz, 1 H), 7.17 (d, *J* = 8.0 Hz, 1 H), 7.01 (dd, *J* = 8.6, 2.4 Hz, 1 H), 6.96 (t, *J* = 8.0 Hz, 1 H), 6.84 (d, *J* = 8.6 Hz, 1 H), 4.39 (tt, *J* = 12.2, 4.1 Hz, 1 H), 4.27 – 4.31 (m, 2 H), 3.72 (s, 3 H), 3.70 (s, 3 H), 2.88 – 2.95 (m, 2 H), 2.23 – 2.31 (m, 2 H), 1.72 – 1.77 (m, 2 H). $^{13}$C-NMR (100 MHz, DMSO-d6) δ ppm 155.3, 153.9, 148.9, 144.4, 134.7, 130.8, 128.3, 123.7, 122.4, 112.6, 112.0, 108.1, 105.8, 101.5, 56.3, 55.8, 51.1, 43.9, 29.1. HRMS (EI): m/z $[M]^+$ Calcd for $C_{21}H_{23}BrN_4O_4$ 474.0903, found: 474.1014.

*4-(4-methoxy-2-oxo-2,3-dihydro-1H-1,3-benzodiazol-1-yl)-N-(3-methoxy-4-methylphenyl)piperidine-1-carboxamide **(TH9525)**.*

The title compound was synthesized according to general procedure H from 4-methoxy-1-(piperidin-4-yl)-2,3-dihydro-1H-1,3-benzodiazol-2-one; 2,2,2-trifluoroacetic acid (20 mg, 0.055 mmol) and 4-methyl-3-methoxyaniline (7.6 mg, 0.055 mmol) which afforded 70% yield. LCMS $[M+H]^+$ 411. $^1$H-NMR (400 MHz, DMSO-d6): δ ppm 11.0 (s, 1H), 8.50 (s, 1H), 7.19 (d, J = 1.6 Hz, 1H), 7.00 (dd, J = 8.1, 1.8 Hz, 1 H), 6.92 – 6.99 (m, 2 H), 6.86 (d, J = 8.0 Hz, 1 H), 6.70 (d, J = 8.1 Hz, 1 H), 4.37 (tt, J = 12.3, 4.1 Hz, 1 H), 4.25 – 4.32 (m, 2 H), 3.84 (s, 3 H), 3.75 (s, 3 H), 2.87 – 2.97 (m, 2 H), 2.202 – 2.33 (m, 2 H), 2.08 (s, 3 H), 1.67 – 1.76 (m, 2 H).

**Supporting Information References**

(1) Michel, M.; Visnes, T.; Homan, E. J.; Seashore-Ludlow, B.; Hedenström, M.; Wiita, E.; Vallin, K.; Paulin, C. B. J.; Zhang, J.; Wallner, O.; Scobie, M.; Schmidt, A.; Jenmalm-Jensen, A.; Warpman Berglund, U.; Helleday, T. Computational and Experimental Druggability Assessment of Human DNA Glycosylases. *ACS Omega* **2019**, *4* (7), 11642–11656. https://doi.org/10.1021/acsomega.9b00162.

(2) Jafari, R.; Almqvist, H.; Axelsson, H.; Ignatushchenko, M.; Lundbäck, T.; Nordlund, P.; Martinez Molina, D. The Cellular Thermal Shift Assay for Evaluating Drug Target Interactions in Cells. *Nat Protoc* **2014**, *9* (9), 2100–2122. https://doi.org/10.1038/nprot.2014.138.

(3) Visnes, T.; Cázares-Körner, A.; Hao, W.; Wallner, O.; Masuyer, G.; Loseva, O.; Mortusewicz, O.; Wiita, E.; Sarno, A.; Manoilov, A.; Astorga-Wells, J.; Jemth, A.-S.;

Pan, L.; Sanjiv, K.; Karsten, S.; Gokturk, C.; Grube, M.; Homan, E. J.; Hanna, B. M. F.; Paulin, C. B. J.; Pham, T.; Rasti, A.; Berglund, U. W.; Nicolai, C. von; Benitez-Buelga, C.; Koolmeister, T.; Ivanic, D.; Iliev, P.; Scobie, M.; Krokan, H. E.; Baranczewski, P.; Artursson, P.; Altun, M.; Jensen, A. J.; Kalderén, C.; Ba, X.; Zubarev, R. A.; Stenmark, P.; Boldogh, I.; Helleday, T. Small-Molecule Inhibitor of OGG1 Suppresses Proinflammatory Gene Expression and Inflammation. *Science* **2018**, *362* (6416), 834–839. https://doi.org/10.1126/science.aar8048.

(4) Parkhurst, J. M.; Winter, G.; Waterman, D. G.; Fuentes-Montero, L.; Gildea, R. J.; Murshudov, G. N.; Evans, G. Robust Background Modelling in DIALS. *J Appl Cryst* **2016**, *49* (6), 1912–1921. https://doi.org/10.1107/S1600576716013595.

(5) Evans, P. Scaling and Assessment of Data Quality. *Acta Crystallogr D Biol Crystallogr* **2006**, *62* (Pt 1), 72–82. https://doi.org/10.1107/S0907444905036693.

(6) Collaborative Computational Project, Number 4. The CCP4 Suite: Programs for Protein Crystallography. *Acta Crystallogr D Biol Crystallogr* **1994**, *50* (Pt 5), 760–763. https://doi.org/10.1107/S0907444994003112.

(7) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser Crystallographic Software. *J Appl Cryst* **2007**, *40* (4), 658–674. https://doi.org/10.1107/S0021889807021206.

(8) Emsley, P.; Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. *Acta Crystallogr D Biol Crystallogr* **2004**, *60* (Pt 12 Pt 1), 2126–2132. https://doi.org/10.1107/S0907444904019158.

(9) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Cryst D* **1997**, *53* (3), 240–255. https://doi.org/10.1107/S0907444996012255.

(10) Visnes, T.; Benítez-Buelga, C.; Cázares-Körner, A.; Sanjiv, K.; Hanna, B. M. F.; Mortusewicz, O.; Rajagopal, V.; Albers, J. J.; Hagey, D. W.; Bekkhus, T.; Eshtad, S.; Baquero, J. M.; Masuyer, G.; Wallner, O.; Müller, S.; Pham, T.; Göktürk, C.; Rasti, A.; Suman, S.; Torres-Ruiz, R.; Sarno, A.; Wiita, E.; Homan, E. J.; Karsten, S.; Marimuthu, K.; Michel, M.; Koolmeister, T.; Scobie, M.; Loseva, O.; Almlöf, I.; Unterlass, J. E.; Pettke, A.; Boström, J.; Pandey, M.; Gad, H.; Herr, P.; Jemth, A.-S.; El Andaloussi, S.; Kalderén, C.; Rodriguez-Perales, S.; Benítez, J.; Krokan, H. E.; Altun, M.; Stenmark, P.; Berglund, U. W.; Helleday, T. Targeting OGG1 Arrests Cancer Cell Proliferation by Inducing Replication Stress. *Nucleic Acids Res*. https://doi.org/10.1093/nar/gkaa1048.