# N-Hydroxyimides and hydroxypyrimidinones as inhibitors of the DNA repair complex ERCC1-XPF

# *N*-Hydroxyimides and hydroxypyrimidinones as inhibitors of the DNA repair complex ERCC1-XPF

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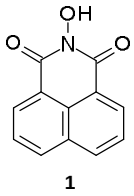
**Abstract**
A high throughput screen allowed the identification of *N*-hydroxyimide inhibitors of ERCC1-XPF endonuclease activity with micromolar potency, but they showed undesirable selectivity profiles against FEN-1. A scaffold hop to a hydroxypyrimidinone template gave compounds with similar potency but allowed selectivity to be switched in favour of ERCC1-XPF over FEN-1. Further exploration of the structure-activity relationships around this chemotype gave sub-micromolar inhibitors with >10-fold selectivity for ERCC1-XPF over FEN-1.

**Keywords:**
DNA repair
ERCC1-XPF
*N*-Hydroxyimide
Hydroxypyrimidinone

Platinum-based chemotherapeutics such as cisplatin result in several forms of DNA damage, however their effectiveness can be limited by efficient DNA repair processes. The ERCC1-XPF complex is essential for one such process, nucleotide excision repair (NER), and there is evidence that it is a good therapeutic target for potential intervention in a range of cancers,[1,2] where inhibition of ERCC1-XPF could enhance the effectiveness of chemotherapeutic agents.

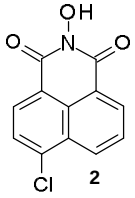
A high-throughput screen using a fluorescence-based *in vitro* biochemical assay[3] allowed the identification of hit compounds **1** and **2** as inhibitors of the endonuclease activity of ERCC1-XPF, with micromolar potency and good ligand efficiency (Figure 1). Despite their flat structures and the *N*-hydroxyimide motif which was considered undesirable, they did not show DNA intercalation of our assay substrate and we noted the similarity of the structure to known flap endonuclease 1 (FEN-1) inhibitors[4] and the natural product flutimide,[5,6] an inhibitor of influenza endonuclease. We therefore sought to explore whether it was possible to gain additional potency and selectivity for ERCC1-XPF over other nucleases including FEN-1 from this starting point. The testing of initial analogues showed that the *N*-hydroxy group was essential for inhibitory activity since replacement of the *N*-OH by either *N*-H or *N*-methyl led to complete loss of activity. The mode of inhibition of FEN-1 by *N*-hydroxyimides has been proposed to involve chelation of metal ions at the endonuclease active site;[4] the enzymatic activity of ERCC1-XPF is also metal-dependent and the presence of $Mn^{2+}$ is required for substrate turnover in the biochemical assay, so it was our hypothesis

that the hits are also able to inhibit ERCC1-XPF through binding to a metal ion at the endonuclease active site.



1

ERCC1-XPF $IC_{50}$ = 30 μM
Ligand efficiency = 0.38
FEN-1 $IC_{50}$ = 0.3 μM



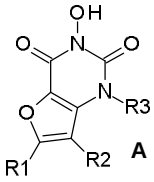
2

ERCC1-XPF $IC_{50}$ = 12 μM
Ligand efficiency = 0.39
FEN-1 $IC_{50}$ = 0.7 μM

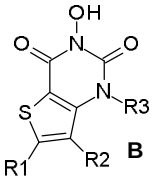
**Figure 1.** Initial hit compounds

Synthesis and testing of known FEN-1 inhibitors **3**-**6**[4] (Table 1) showed that they could also inhibit ERCC1-XPF at micromolar levels, although due to their high potency against FEN-1 they were highly selective for this target versus ERCC1-XPF. In order to monitor the selectivity of inhibitors during the optimization process, we employed counter screen assays against the non-structure specific nuclease DNase I as well as FEN-1. The phenyl analogue **7** showed lower potency than the thiophene **5**, although the addition of aniline (**8**) or pyrrolidine (**9**) substituents allowed some potency to be regained. The acetamido- substituted positional variants (examples **10**-**12**) all showed similar potency against ERCC1-XPF but encouragingly suggested that it might be possible to tune the selectivity of these inhibitors towards ERCC1-XPF inhibition and away from FEN-1 (**11**, **12**), although it still remained in favour of FEN-1. The *N*-substituted analogue **13** was consistent with **6** in suggesting that substitution at this position is not tolerated, and **14** showed good potency for its size although was still more potent against FEN-1.

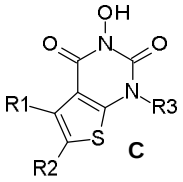
**Table 1.** Initial activity and selectivity data based around known *N*-hydroxyimide FEN-1 inhibitors and their analogues



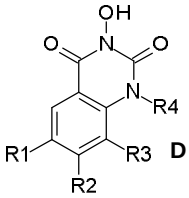
A



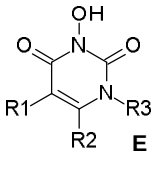
B



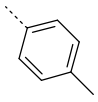
C

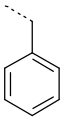
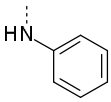
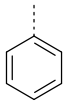


D



E

| Compound | Core | R1 | R2 | R3 | R4 | ERCC1-XPF $IC_{50}$/ μM | FEN-1 $IC_{50}$/ μM | DNase I $IC_{50}$/ μM |
|---|---|---|---|---|---|---|---|---|
| **3** | A | H |  | H | - | 1.5 | 0.057 | >100 |
| **4** | B | H |  | H | - | 1.8 | 0.003 | >100 |
| **5** | C | H | H | H | - | 3.9 | 0.009 | >100 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **6** | C | H | H |  | - | 80.4 | 0.68 | >100 |
| **7** | D | H | H | H | H | 18.1 | 0.30 | >100 |
| **8** | D | H |  | H | H | 3.9 | 0.23 | 30.2 |
| **9** | D | H |  | H | H | 9.1 | 1.1 | 27.9 |
| **10** | D | H | H |  | H | 6.1 | 0.038 | >100 |
| **11** | D | H |  | H | H | 6.4 | 2.1 | >100 |
| **12** | D |  | H | H | H | 6.6 | 2.0 | 87 |
| **13** | D | H | H | H | Me | 96.2 | 2.1 | >100 |
| **14** | E | H |  | H | - | 1.5 | 0.63 | >100 |

In order to advance the series with the aim of improving potency and selectivity we investigated a scaffold hop to introduce additional vectors for exploration and avoid the requirement for the N-O bond, which was considered a liability from an ADMET perspective. The scaffold chosen was hydroxypyrimidinone (Figure 2), which is known to be able to bind metal ions (typically $Mg^{2+}$) in a similar way to the *N*-hydroxyimide and is present in the blockbuster HIV integrase drug raltegravir,[7] inhibitors of HCV polymerase[8,9] and RNase H.[10]

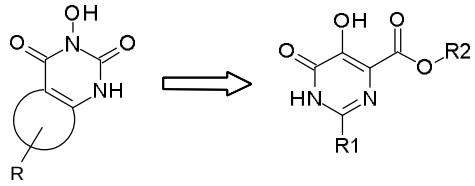
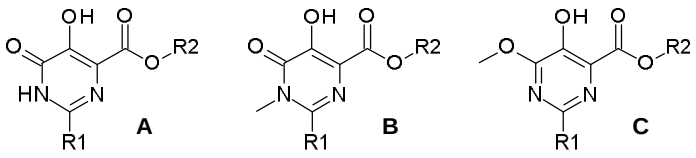


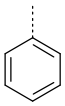
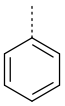
**Figure 2.** Scaffold hop to hydroxypyrimidinone motif[11]

Compounds containing this scaffold were synthesized and tested, and gratifyingly initial examples showed comparable inhibition of ERCC1-XPF to the *N*-hydroxyimides (Table 2). With either thiophene or phenyl as the R1 substituent, the carboxylic acids **15** and **17** showed $IC_{50}$ values around 3 μM and approximately 4-fold selectivity for ERCC1-XPF over FEN-1. Their methyl ester analogues **16** and **18** were found to have weaker potency than the corresponding carboxylic acids and modifications around the metal-binding motif, through

either *N*-methylation (**19**) or *O*-methylation (**20**), led to losses in potency. These initial examples represented a promising start with respect to potency against ERCC1-XPF and achieving selectivity against FEN-1 and DNase I.

**Table 2.** Initial activity data from scaffold hop

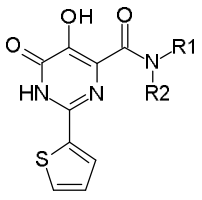


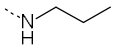
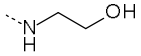
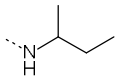
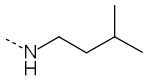
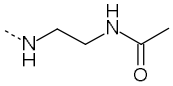
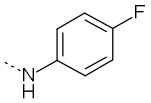
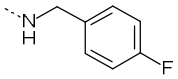
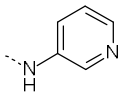
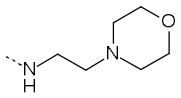

| Compound | Core | R1 | R2 | ERCC1-XPF $IC_{50}$/ µM | FEN-1 $IC_{50}$/ µM[a] | DNase I $IC_{50}$/ µM[a] |
|---|---|---|---|---|---|---|
| **15** | A |  | H | 2.9 | 10.4 | >100 |
| **16** | A |  | Me | 27.3 | 10.1 | >100 |
| **17** | A |  | H | 3.9 | 16.4 | >100 |
| **18** | A |  | Me | 16.4 | 53.4 | >100 |
| **19** | B |  | H | 48.8 | 88.9 | >100 |
| **20** | C |  | H | >100 | nt | nt |

[a] nt = not tested.

The carboxylic acids and esters are highly polar and carry limited vectors for additional substitution for exploring potency and selectivity gains, so amides instead of acids were investigated (Table 3). It was found that a range of amides was well tolerated and potency gains to the sub-micromolar level against ERCC1-XPF were achieved, although the SAR appeared relatively flat in this region. The primary carboxamide **21**, methyl amide **22**, small lipohilic alkyl chains (**23**, **26**, **27**) and appended polar groups (**24**, **28, 32**) were all well tolerated, but the tertiary amide **25** was less potent. Aryl and heteroaryl groups also showed similar potency (**29**, **31**). They showed variable FEN-1 selectivity but in the best case, with the primary carboxamide **21**, greater than 20-fold selectivity was observed.

**Table 3.** Activity data from variations at amide position



| Compound | NR1R2 | ERCC1-XPF $IC_{50}$/ µM | FEN-1 $IC_{50}$/ µM | DNase I $IC_{50}$/ µM[a] |
|---|---|---|---|---|
| **21** |  | 1.0 | 23.3 | >100 |
| **22** |  | 0.6 | 12.0 | 67.6 |
| **23** |  | 2.7 | 4.2 | >100 |
| **24** |  | 0.7 | 4.0 | >100 |
| **25** |  | 4.6 | 13.7 | nt |
| **26** |  | 0.6 | 1.3 | >100 |
| **27** |  | 0.5 | 3.5 | nt |
| **28** |  | 0.8 | 5.0 | 62.6 |
| **29** |  | 0.8 | 10.5 | nt |
| **30** |  | 4.2 | 2.1 | >100 |
| **31** |  | 0.7 | 8.6 | >100 |
| **32** |  | 1.0 | 4.1 | 58.9 |

[a] nt = not tested.

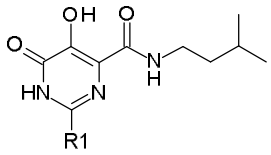
Although the potency gains from introducing the amides were modest relative to the carboxylic acids, a better balance in compound properties and ADMET profiles was obtained (Table 4). While the carboxylic acids typically showed log D values of less than zero and were not permeable in the PAMPA assay, modifying the amide substituent enabled tuning of the log D of the compounds which led to corresponding improvements in the permeability. They also displayed good stability in both mouse and human liver microsomes.

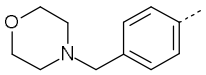
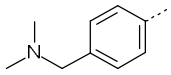
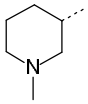
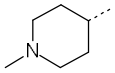
**Table 4.** Measured *in vitro* ADMET profiles for selected compounds

| Compound | Log D | Cytotox Hep-G2 v-50/ µM | MLM $t_{1/2}$/ mins | HLM $t_{1/2}$/ mins | PAMPA $P_{app}$/ $nms^{-1}$ |
|---|---|---|---|---|---|
| **21** | -0.4 | >10 | 150 | 139 | 7 |
| **22** | 0.5 | >10 | >400 | 181 | 31 |
| **26** | 2.1 | >10 | >400 | >400 | 97 |

With the isopentyl group in place as the amide substituent, changes at the pyrimidinone 2-position were explored (Table 5). In this region it was found that pendant groups could be added to a phenyl ring without significant losses in potency, such as basic groups in **33** and **34**, but the introduction of saturated ring systems led to losses in potency (examples **35**-**38**).

**Table 5.** Activity data from variations at 2-position




| Compound | R1 | ERCC1-XPF $IC_{50}$/ µM | FEN-1 $IC_{50}$/ µM |
|---|---|---|---|
| **33** |  | 0.8 | 38.1 |
| **34** |  | 1.7 | 71.3 |
| **35** |  | 6.3 | >100 |
| **36** |  | 8.4 | >100 |
| **37** |  | 25.3 | >100 |
| **38** |  | 6.9 | >100 |

In order to address the possibility that the compounds could be inhibiting enzyme activity in a non-specific manner, such as through general metal chelation, it was important to test the compounds in biophysical assays to confirm binding to ERCC1-XPF. Compound **22** was profiled by surface plasmon resonance (SPR), where it displayed a $K_D$ of 4 µM with fast binding kinetics (Figure 3) and also in a thermal shift assay, where it showed a $\Delta T_m$ of 4°C.

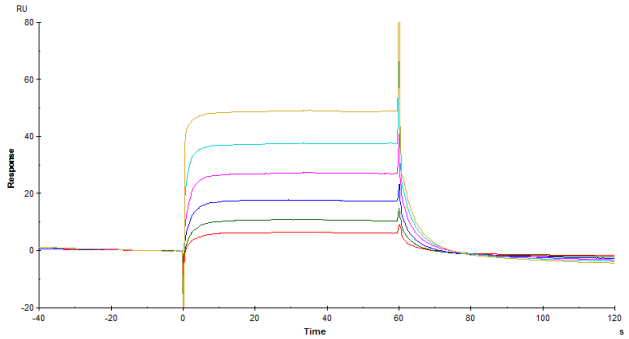



**Figure 3.** SPR sensorgram for compound **22**

Compound **22** was tested (up to 10 µM) in cell based assays using A375 melanoma cells to assess effects on DNA repair. Three assays were used to measure the following parameters: direct nucleotide excision repair (NER) using a recombinant GFP reporter assay,[3] potentiation of cisplatin induced cell death and a high content imaging assay measuring levels of γH2AX foci, a marker of DNA repair.[12] We did not observe any inhibition of repair of damaged GFP DNA by compound **22** in the NER assay and in the cell viability assay run over 5 days, the compound alone did not cause toxicity but neither did it enhance the cell death seen with cisplatin (dose response range of cisplatin of 0.1 to 3 µM). Similarly, we did not observe a delay in the repair of DNA damage caused by cisplatin by addition of compound **22** in the γH2AX foci assay (data not shown). In view of the cell activity shown by a compound with similar biochemical potency,[12] it is somewhat surprising that **22** showed no activity in these assays and there are a number of reasons why this might be the case. Despite acceptable physical and ADMET properties, it might be that insufficient compound concentration at the target was achieved, or alternatively that inhibition of ERCC1-XPF activity in these cells is not sufficient to stop DNA repair. Unlike the catechol series,[12] no cell toxicity was observed with compound alone for the hydroxypyrimidinones, which could suggest that the cellular effects seen with the catechol series are due to inhibiting targets and pathways in addition to ERCC1-XPF. Further studies to measure target engagement in the cell and probe on- versus off-target effects for both the catechols and hydroxypyrimidinones would be required to confirm this, but it might be the case that a combination approach will be required to achieve efficacy in a therapeutic.

In summary, we have shown that compounds with the *N*-hydroxyimide metal-binding chemotype are able to inhibit the activity of ERCC1-XPF, although they are also highly potent against FEN-1. Promisingly, performing a scaffold hop to a hydroxypyrimidinone template allowed sub-micromolar inhibitors of ERCC1-XPF to be obtained with >10-fold selectivity over the other nucleases FEN-1 and DNase I; the inhibitors were also shown to bind to ERCC1-XPF in SPR and thermal shift assays. Further work to understand the binding mode of these compounds, for example if a crystal structure could be obtained, might reveal additional opportunities to drive improvements in enzyme affinity and cell activity and advance their utility as probes for ERCC1-XPF biology.

**Acknowledgements**
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**References and notes**


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