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Corrected structure of mirin, a small molecule inhibitor of the Mre11-Rad50-Nbs1 complex

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To the editor

The Mre11/Rad50/Nbs1 (MRN) proteins form a complex that plays a critical role in the response of cells to DNA damage. The complex functions to regulate the DNA damage recognition protein ataxia telangiectasia mutated (ATM). Because defects in MRN enhance radiosensitivity, it has been proposed that small molecule inhibitors targeted to these proteins might be used as radiosensitizers in the clinical arena. To this end, Dupre *et al.* recently identified in a high-throughput screen, a novel inhibitor of Mre11, called mirin, which was shown to prevent MRN-dependent activation of ATM and to inhibit Mre11-associated exonuclease activity¹. While attempting to repeat the work of Dupre *et al.*, we found that the published structure of mirin was incorrect. We now report the correct structure for this compound.

Mirin was identified from a chemical screen of a 10,000 compound library from Chembridge Corporation as compound # 6451023 and was described as 6-(4-hydroxyphenyl)-2-thioxo-2,3-dihydro-4(1H)-pyrimidinone (Fig. 1a, compound **1**). We obtained 3 mg of 6451023 from Chembridge and confirmed that it had biological activity in our assays; in particular, it induced G₂ arrest in MDA-MB-231 cells similar to that observed by Dupre *et al.*¹. To extend these studies, we synthesized more of compound **1** from thiourea and methyl 4-hydroxybenzoylacetate according to a modified literature procedure². The product of this synthesis was white, unlike the orange compound obtained from Chembridge and it failed to induce G₂ arrest in our bioassay. The NMR spectra of **1** were consistent with the spectra of analogous 6-arylthiouracil derivatives² but differed from those reported by Dupre *et al.*¹. There remained inadequate quantity of compound 6451023 to perform a comparative NMR, but we were able to obtain a mass spectrum showing the molecular ion of 221 [M+H⁺] which was consistent with the published structure.

Discussion with Chembridge led to the suggestion that the original compound they had provided was 5-(4-hydroxybenzylidene)-2-thioxo-4-imidazolidinone (compound **2**). We synthesized compound **2** by condensing 2-thiohydantoin with 4-hydroxybenzaldehyde according to a published procedure³ and found it to be yellow rather than orange with NMR spectra inconsistent with that shown by Dupre *et al.* However, the NMR spectra of the synthesized compound **2** was consistent with the NMR spectra of closely related 5-arylidene-2-thiohydantoin derivatives⁴. We concluded that the product of our synthesis did indeed have the structure shown for compound **2**, but it also exhibited no activity in our bioassay.

Further discussion with Chembridge led to the suggestion that the original compound might be 5-(4-hydroxyphenylidene)-2-imino-1,3-thiazolidin-4-one (compound **3**). As with all these isomeric compounds **1–3**, the molecular weight would be identical. We synthesized **3** from 4-hydroxybenzaldehyde and pseudothiohydantoin using a published procedure³ and obtained an orange compound with the same NMR spectra as originally published by Dupre *et al.* and consistent with NMR data on related 5-arylidene-pseudothiohydantoins^{5,6}. Furthermore, we find that it has the same biological activities as the original compound reported by Dupre *et al.*, namely induction of G₂ arrest and inhibition of radiation-induced phosphorylation of ATM (Fig. 1b,c). Hence, we conclude that **3** is the correct structure of mirin.

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Figure 1.

(a) The structures of compounds discussed in the text; compound 3 is the corrected structure for mirin. (b) Mirin causes a G₂ arrest. MDA-MB-231 cells were incubated with or without 100 μ M Mirin for 24 h, then fixed, stained with propidium iodide and analyzed by flow cytometry. (c) Mirin inhibits the phosphorylation of ATM. MDA-MB-231 cells were incubated with mirin for 1 h, irradiated (10 Gy) and cell lysates made 30 min later. Western blots were probed for ATM (Abcam) and p(S1981)ATM (Epitomics). Panels b and c should be compared to similar experiments of Dupre *et al.*¹.