Enlightening the reaction mechanism of furimazine oxidation in DMSO

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Amongst the enormous variety of luciferins, imidazopyrazinone family of substrates stands out as one of the most well-known since it includes the substrates of some of the most widely employed luciferases, including NanoLuc, a small artificial luciferase engineered from the active subunit of the *Oplophorus gracilirostris* luciferase [1]. The imidazopyrazinonic-core substrate which undergoes the reaction is a coelenterazine analogue called furimazine. The light emission in this system is supposed to be the result of the formation of a cyclodioxetanonic intermediate which decomposes, with the elimination of a CO_2 molecule, to produce an amide in its singlet excited state. Understanding the reaction mechanism of furimazine oxidation is crucial to help the identification of the emitting species. In this study, we investigated the formation of the furimazine dioxetanonic intermediate and its decomposition leading to the formation of the excited state furimamide, responsible for light emission. The reaction path was evaluated in implicit dimethylsulfoxide (DMSO), a common polar solvent used in experimental studies of substituted imidazopyrazinone bioluminescence.

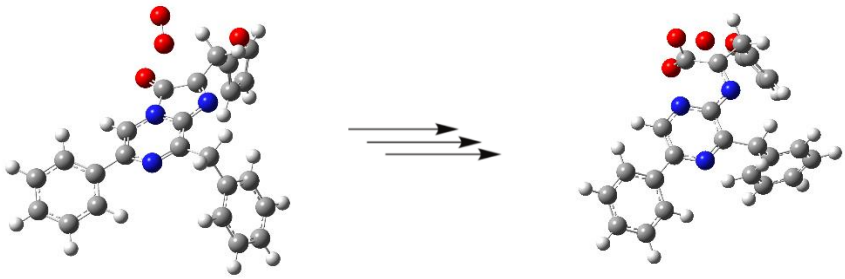

Figure 1 : Oxygenation of imidazopyrazinone core of furimazine to give the corresponding dioxetanone

The results are coherent with the expectations, showing a pathway similar to the one proposed for coelenterazine disulfonate [2], characterized by low energy barriers. The decomposition of the dioxetanone has been studied for three different forms of it, the anionic one and two neutral ones - each of them being the precursor of a different form of furimamide - in order to verify whether it's feasible or not to obtain those different structures as products. Overall, our computational modeling provides valuable insights into the oxidation mechanism of furimazine in DMSO, offering a deeper understanding of its chemiluminescent properties and guiding the further steps of the research towards the exploration of the catalytic mechanism in the protein environment.

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[2] Ding B.-W., Liu Y.-J., *J. Am. Chem. Soc.,* **2017**, 139, 1106-1119