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Excited-State Proton Transfer Can Tune the Color of Protein Fluorescent Markers

Daiana T. Mancini*, Tamiris M. Assis, Teodorico C. Ramalho, Kakali Sen, Mario Barbatti, Walter Thiel

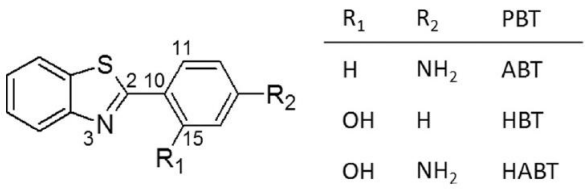
*Department of Chemistry, Federal University of Lavras, 37200-000 Lavras, Brazil.

daianateixeira60@yahoo.com.br

Abstract: Phenylbenzothiazole (PBT) compounds exhibit antitumor properties and are highly selective in vitro and in vivo. Their antitumor action relies on the docking to tyrosine kinase (TKs),[1,2] enzymes that are usually over-expressed in several types of carcinomas.[3] A number of PBT compounds and complexes [4] strongly interact with the active site of kinases. The original PBT lead compound [2-(4'-aminophenyl)benzothiazole (ABT)] (Scheme 1) exhibits nanomolar activity against certain human breast cancer cell lines in vitro. [1] With regard to the goals of this work, it is particularly interesting that diverse (hydroxyphenyl)benzothiazole (HBT) compounds also have potent antitumor activity in human breast and colon cancer cell lines. In the physical chemistry community, the HBT isomer 2-(2'-hydroxyphenyl)benzothiazole is well known for its strong Stokes shift caused by excited-state intramolecular proton transfer (ESIPT) [5,6]. HBT undergoes ESIPT in the gas phase and in aprotic solution (Figure 1). Upon photoexcitation, the first singlet excited state (S1) of the enol form is populated. Then, a keto tautomer is formed by ultrafast proton transfer in the S1 state.[19] Because ESIPT is much faster (30–50 fs) than radiative decay (~106 fs), any observed fluorescence is normally due to the keto tautomer. However, depending on the environment, ESIPT in HBT can be partially or completely inhibited, which will increase the fluorescence quantum yield of the enol species. [6,7] ESIPT can also be followed by other relaxation processes affecting fluorescence intensities: intersystem crossing (ISC) with formation of the keto tautomer in the triplet ground state (T1) or isomerization of the cis- to the trans-keto form, which will proceed through a S1/S0 crossing region and may thus lead to internal conversion (IC). [6] This scenario opens the possibility of creating two-color fluorescent markers. Aiming at combining the tumor selectivity of ABT and the ESPT properties of HBT, we chose to study HABT, which differs from ABT by a hydroxyl group at the R1 position (Scheme 1). Our hypothesis is that the proportions and intensities of violet and green emissions from HABT depend on the protein active site conformation, which modulates the rates of ESIPT, ISC, and IC. Thus, changes in the fluorescence spectrum of HABT bound to TK could be the basis for a new method to detect mutations in cancer cells, usually associated to development of drug resistance. We show by quantum mechanical/molecular mechanical (QM/MM) simulations that phenylbenzothiazoles undergoing an excited-state proton transfer (ESPT) can be used to probe protein binding

sites. For 2-(2'-hydroxy-4'aminophenyl)benzothiazole (HABT) bound to a tyrosine kinase, the absolute and relative intensities of the fluorescence bands arising from the enol and keto forms are found to be strongly dependent on the active site conformation. The emission properties are tuned by hydrogen-bonding interactions of HABT with the neighboring amino acid T766 and with active-site water. The use of ESPT tuners opens the possibility of creating two-color fluorescent markers for protein binding sites, with potential applications in the detection of mutations in cancer cell lines.

Key-words: Phenylbenzothiazole; fluorescence; QM/MM



R_1	R_2	PBT
H	NH_2	ABT
OH	H	HBT
OH	NH_2	HABT

Scheme 1. ABT, HBT, and HABT

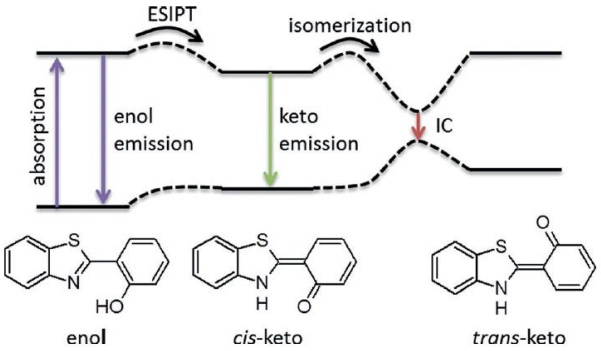


Figure 1. Schematic photophysics of HBT.

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References:

[1] S. Tzanopoulou, *et al*. J. Med. Chem. 53, 4633 (2010).
[2] J. W. Hicks, H. F. VanBrocklin, A. A. Wilson, S. Houle, N. Vasdev, Molecules 15, 8260 (2010).
[3] H. Daub, K. Specht, A. Ullrich, Nat. Rev. Drug Discovery 3, 1001 (2004).
[4] D. T. Mancini, E. F. Souza, M. S. Caetano, T. C. Ramalho, Magn. Reson. Chem. 52, 129 (2014).
[5] J. Zhao, S. Ji, Y. Chen, H. Guo, P. Yang, Phys. Chem. Chem. Phys. 14,8803 (2012).
[6] M. Barbatti, A. J. A. Aquino, H. Lischka, C. Schriever, S. Lochbrunner, E. Riedle, Phys. Chem. Chem. Phys. 11, 1406 (2009).
[7] Y. H. Kim, *et al.*, Photochem. Photobiol. Sci. 9, 722 (2010).