Synthesis and properties of a new benzamide-containing nitrobenzoxadiazole endowed with high stability to metabolic hydrolysis

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The antitumor agent 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)thio)hexan-1-ol (**1**) is a potent inhibitor of GSTP1-1, a glutathione *S*-transferase capable of inhibiting apoptosis by binding to JNK1 and TRAF2. We recently demonstrated that, unlike its parent compound, the benzoyl ester of **1** (compound **3**) exhibits negligible reactivity towards GSH, and has a different mode of interaction with GSTP1-1[1]. Unfortunately, **3** is susceptible to rapid metabolic hydrolysis, by esterases. In an effort to improve the metabolic stability of **3**, its ester group has been replaced by an amide, leading to N-(6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)thio)hexyl)benzamide (**4**). Unlike **3**, compound **4** was stable to human liver microsomal carboxylesterases, but retained the ability to disrupt the interaction between GSTP1-1 and TRAF2 regardless of GSH levels. Moreover, **4** exhibited both a higher stability in the presence of GSH and a greater cytotoxicity towards cultured A375 melanoma cells, in comparison with **1** and its analogue **2**. Compound **4** interaction with GST can have a twofold effect in contrasting tumors: it inhibits the detoxifying activity of GST, thus increasing the half-life of some chemotherapeutic drugs, and it affects GST-TRAF2 interaction, leading to apoptosis. In the light of this rationale our present findings suggest that **4** deserves further preclinical testings.

[1]C. Fulci, J Enzyme Inhib Med Chem. 32(1) (2017) 240 -247.

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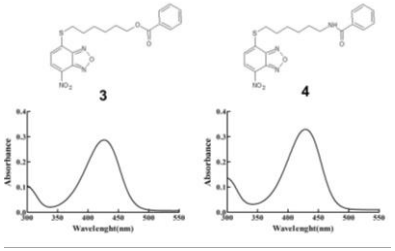

Figure 1 Structures and UV–visible spectra of **3** and **4** (20 μM), dissolved in 0.1M K-Phosphate Buffer with 0.1 mM EDTA and 0.1%Triton X-100, pH 7.4, recorded at 25°C.

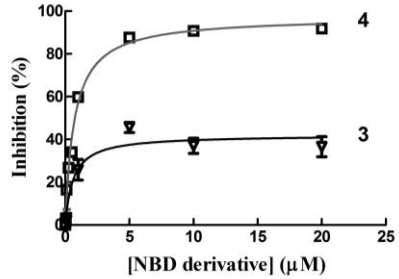

Figure 2.. Inhibion of GTP1-1 conjugation activity by **3** and **4** at 25 °C.

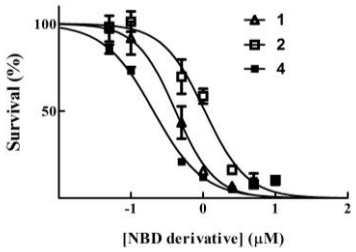

Figure 3. Cytotoxicity dose–response curves were obtained on human melanoma A375 cells treated with varying concentrations of compounds **1**, **2** and **4**. Cell growth was evaluated by the SRB assay after 48 h of treatment.

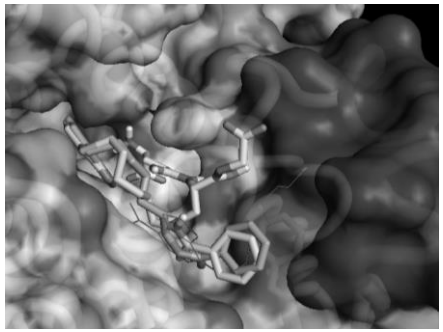

Figure 4. Protein–ligand docking: best binding poses for **3** and **4**. Both compounds show a similar arrangement, with a shifted NBD moiety and the benzoyl terminal making extensive contact with Phe 8, limiting the mobility of H2 region (dark surface). GSH is also shown in sticks representation.