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COMMUNICATION

3-Benzoylquinoxalinone as photoaffinity labelling derivative with fluorogenic properties allowing reaction monitoring under “no-wash” conditions

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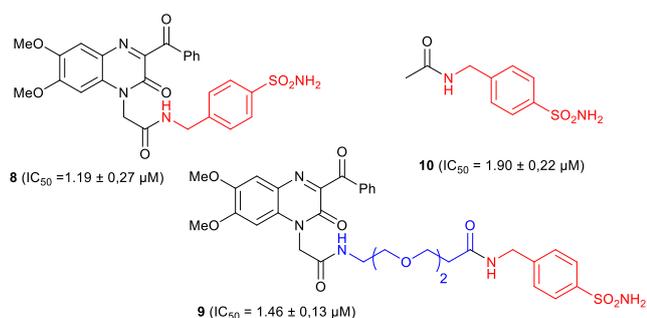
Described herein is a quinoxalinone-based photoaffinity probe with caged fluorescence properties. Upon visible blue LED irradiation (λ_{max} 450 nm), this photo-crosslinker is able to covalently capture proteins with concomitant fluorescence labelling. This process enables monitoring applications under “no wash” conditions.

The identification of protein-protein interactions or of biological targets of small-molecule ligands are of prime interest in medicinal chemistry for elucidating new mode of actions of pharmacophores. Important applications are also found in chemical biology for fundamental understanding of cellular functions, or in structural biology for identifying amino acid residues of proteins in contact with ligands.¹

Methods for identifying biological targets include *in vitro* activity assays, affinity chromatography,² activity-based probe (ABP) technology,³ or photoaffinity labelling (PAL) approach.⁴ Whilst the two first approaches rely on the use of isolated proteins or crude cell lysates, in the two latter strategies, interactions from native environments could also be trapped for their subsequent identification. On one hand, the activity-based probes consist of 1) an affinity linker or ligand that favour interactions with the biological target; 2) an electrophilic warhead (such as an epoxide, a Michael acceptor, an activated halogen derivative, or a quinone methide) that reacts covalently and specifically with nucleophilic residues of the active site of the protein target; and 3) a reporter tag such as a fluorophore for identification and purification of modified proteins. This strategy requires finding a fine tuning of the respective reactivity of the two complementary reactive groups, in order to both increase the chance of covalent bond formation once in close proximity, and decrease the signal to noise ratio (if the electrophile reacts with other nucleophilic derivatives). On the other hand, in the affinity labelling (PAL) strategy, the electrophilic warhead is replaced by a more stable photoreactive function which unveiled highly reactive and transient species only upon triggered UV irradiation. These intermediates are able to react covalently with a larger range of residues, not limited to nucleophilic amino acids. As a direct consequence, this strategy has a broader applications spectrum, while avoiding the biased reactivity of ABP towards specific proteins equipped with the required nucleophilic moieties. Traditional photoreactive functions mostly consist of

benzophenone, aryl azide, and diazirine group that generate diradical, nitrene, and carbene intermediate, respectively, when exposed to 280–365 nm UV irradiation. The access to functionalized chemical probes bearing a photoreactive function, a ligand, and a fluorophore is synthetically challenging, and the presence of a bulky fluorophore may disrupt protein-ligand interactions, and generally low photo-crosslinking yields, which further complicates the isolation and identification of the photo-crosslinked species. To address this point, minimalist probes were recently designed, in which the fluorophore was elegantly replaced by a discrete bioorthogonal handle (*i.e.* terminal alkyne, cyclopropene), that was used to install the fluorescent tag once the photocrosslinking of ligand-protein interactions was achieved.^{5–7} However, this strategy requires a two-step protocol: 1) the photolabelling step to ensure the integrity of the ligand-protein complex during its isolation; 2) a bioorthogonal reaction for installing the fluorescent tag. This approach can also produce background fluorescence due to the presence of residual fluorescent tag typically used in excess, which prevents, among others, real-time monitoring applications. An alternative, yet underexplored approach, consists in using fluorogenic photoreactive functions which are able to both photocrosslink and unveil a fluorescent scaffold in a single-step process. This approach has been combined with diazo,⁸ azide^{8–11} and diazirine-based photoreactive functions.^{12–13} This strategy was extended to the photoreactive diaryltetrazole function, which has shown to preferentially react with aspartic acid and glutamic acid residues of proteins through the formation of a nitrile imine intermediate.¹⁴ However, 280 and 300 nm UV irradiation are required to photoactivate aryl azides and diaryltetrazoles, respectively, which can induce severe damage to biological systems.

used as a model target protein, was investigated. From quinoxalinone **5**, a two-step ester hydrolysis/amidation sequence was carried out to introduce the 4-sulfamoyl benzylamine ligand, which belongs to a well-established family of CA-II inhibitors, leading to the probe **8**.²⁰ In order to investigate the influence of the chain length on the labelling efficiency, probe **9**, in which the ligand is separated by two PEG units from the photoreactive quinoxalinone scaffold, was also accordingly prepared (Scheme 2, see ESI for experimental details).



Scheme 2 Preparation of modified quinoxalinones **8** and **9**.

These compounds in hands, their IC_{50} values were determined, and found to be $1.19 \mu\text{M}$ and $1.46 \mu\text{M}$ for **8** and **9**, respectively. These values are in agreement with the one found for the parent compound benzylamide **10** ($IC_{50} = 1.90 \mu\text{M}$), thus demonstrating that the 3-benzoylquinoxalinone scaffold does not significantly interfere with the binding properties of **10**, whatever the chain length. The photo-crosslinking and concomitant fluorescence labelling of bovine CA-II ($10 \mu\text{M}$) with probes **8** and **9** was conducted in PBS pH 7.4 under visible blue LED irradiation (λ_{max} 450 nm). The photoaffinity labelling could be followed by fluorescence spectroscopy (λ_{ex} 375 nm) under “no-wash” conditions, that is, without filtering the excess of unreacted probe. In fact, no fluorescence increase was observed when the 3-benzoylquinoxalinone is irradiated for 1 h in PBS pH 7.4 (Figure S12). In contrast, as shown in Figure 3A-a and -b, probes **8** and **9** showed a noticeable 10- and -3 fold increase, respectively, in fluorescence at 460 nm upon binding to the protein. The labelling efficiency with probe **8** was found to be about 2% (Figure S19). Most of the labelling was achieved after 15 min and 3 min of irradiation with visible blue LED (λ_{max} 450 nm) and under near UV LED (λ_{max} 405 nm), respectively (Figures S14-16).

Control experiments carried out with **3** containing only the photoreactive part of the probe (Figure 3A-c), or with probe **8** in the presence of CA-II inhibitor, acetazolamide (10 equiv., Figure 3A-d), showed, however, no significant fluorescence increase. These results indicate the specificity of the labelling. Of note, two emission peaks at 460 and 560 nm were observed for quinoxaline derivatives in aqueous systems. Complementary photophysical studies suggest that one of the two peaks may correspond to that of aggregates of quinoxalinone molecules. In fact, only one fluorescence emission band was observed in the organic solvent DMSO, in which quinoxalinones are well soluble (Figure S8).

Moreover, gel electrophoresis analyses further confirmed the fluorescence labelling of CA-II specifically with **8** and under light irradiation stimulus, with the presence of a fluorescent band in the 30 kDa region (Figure 3B, lane 2).

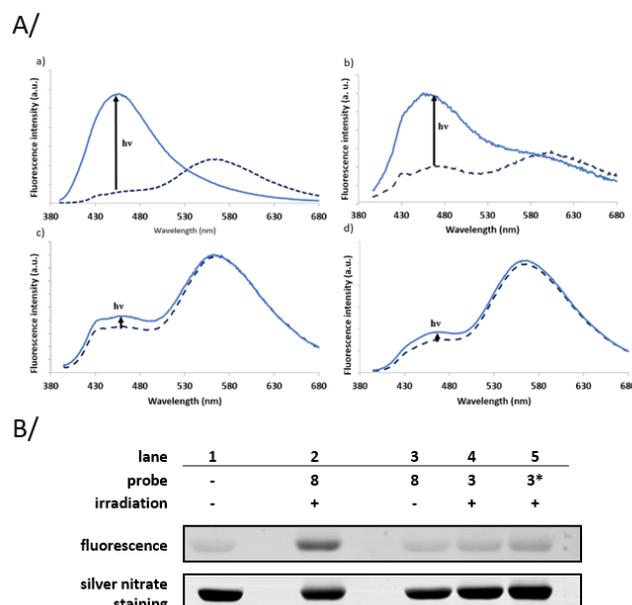


Fig. 3 A/ Fluorescence emission spectra of a mixture of CA-II ($10 \mu\text{M} = 0.3 \text{ mg/mL}$) with different probes ($10 \mu\text{M}$, 1 eq.) (λ_{ex} 375 nm) in water at 25°C , before (dashed line) and after (solid line) 1 h of blue LED light irradiation: (a) probe **8**; (b) probe **9**; (c) quinoxalinone **3**; (d) 30 min pre-incubation with known CA-II inhibitor acetazolamide ($100 \mu\text{M}$, 10 eq.) before introducing probe **8**. **B/** SDS-PAGE analysis of CA-II (30 kDa). Conditions: **8** ($10 \mu\text{M}$), **3** ($10 \mu\text{M}$), CA-II ($10 \mu\text{M} = 0.3 \text{ mg/mL}$) in water and LED blue light irradiation (1 h). *30 min pre-incubation with known CA-II inhibitor acetazolamide ($100 \mu\text{M}$, 10 eq.) before introducing probe **8** ($10 \mu\text{M}$). In-gel fluorescence imaging ($\lambda_{\text{ex}} = 302 \text{ nm}$, $\lambda_{\text{em}} > 535 \text{ nm}$).

The labelling specificity was further investigated by tagging the bovine CA-II mixed with human blood plasma (HBP, Figure 4). Similarly, CA-II was efficiently fluorescently labelled by probe **8**, while only light fluorescence for CA-II was detected with the non-specific probe **3**. In the absence of irradiation, no labelling was observed with **8**, confirming this probe provides spatial and temporal control over reactivity.

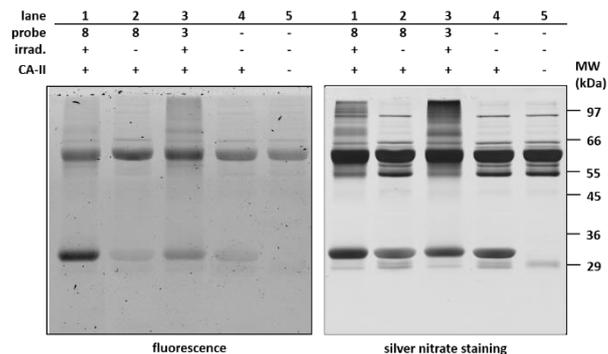
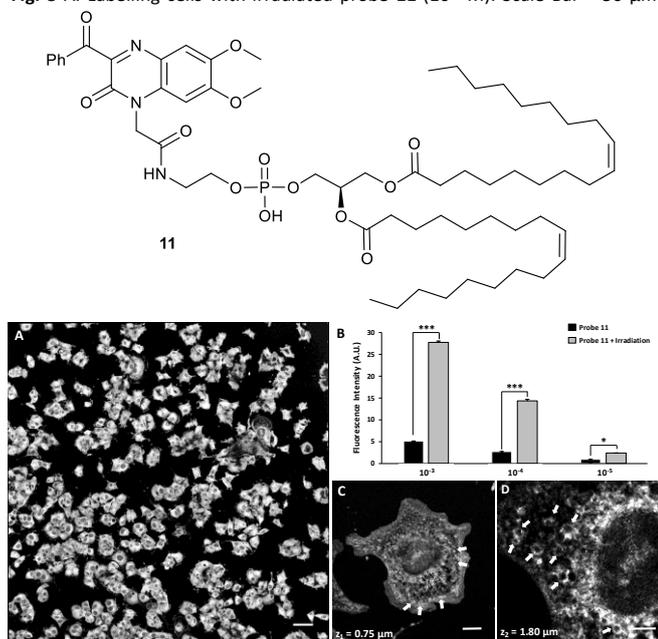


Fig. 4 Specific fluorescence labelling of CA-II (30 kDa) by **8** in the presence of human blood plasma. Conditions: **3** ($100 \mu\text{M}$) or **8** ($100 \mu\text{M}$), CA-II ($100 \mu\text{M} = 3 \text{ mg/mL}$), human blood plasma diluted by 10 in PBS pH 7.4 and visible blue LED light irradiation (2 h). In-gel fluorescence imaging ($\lambda_{\text{ex}} = 302 \text{ nm}$, $\lambda_{\text{em}} > 535 \text{ nm}$).

Encouraged by these results, the fluorescence turn-on properties of quinoxalinones was next investigated in a more complex medium, through the fluorescent labelling of cells under “no-wash” conditions. To illustrate that proof-of-principle, the functionalizable quinoxalinone **5** was equipped with a phospholipid, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine known to interact with biological membranes, yielding phospholipid-based probe **11**, in order to specifically visualize lipid-rich organelles that are abundant in cells (Figure 5).^{21,22} After incubation of fixed cells with probe **11** and visible blue LED irradiation, both for 1 h, confocal microscopy showed a concentration-dependent effective labelling of most, if not all, PC12 cells (Figure 5). Satisfyingly, significantly lower fluorescence was observed in the control experiment carried out in the absence of irradiation (Figure 5B). In addition, higher magnifications revealed subcellular positive elements suggesting that endomembranes around the nuclear membrane as well as membranes of intracellular vesicles are labelled with probe **11**.

Fig. 5 A. Labelling cells with irradiated probe **11** (10^{-3} M). Scale Bar = 50 μ m B.



Influence of probe concentration. *** $p < 0.001$, statistical significance versus probe **11** without irradiation; * $p < 0.05$ statistical significance versus probe **11** without irradiation. C,D. Confocal images at different z position (z_1 and z_2). Scale Bar = 10 μ m (C), Scale Bar = 5 μ m (D). White arrows indicate labelled membrane of intracellular vesicles.

In summary, an easy photolabelling procedure in the presence of compact quinoxalinone derivatives playing the role of both the photoreactive function and pro-fluorophore, is reported. Advantageously, the labelling proceeds effectively under visible blue LED, conditions that are less photo-damaging to proteins and cell contents than traditionally used UV irradiations. The use of carbonyl-conjugated quinoxalinone systems possessing photocrosslinking ability accompanied with caged-fluorescence properties enables direct monitoring of the fluorescence labelling, that is, without removing the excess of unreacted probe. This was leveraged for the fluorescent labelling of endomembrane systems of cells by using a photoreactive phospholipid probe. This proof-of-concept being

established, 3-benzoylquinoxalinone-based probes will be used for identifying new protein-protein interactions occurring in specific subcellular organelles, and results will be published in due course.

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Conflicts of interest

“There are no conflicts to declare”.

Notes and references

- E. L. Vodovozova, *Biochem (Mosc)*, 2007, **72**, 1-20.
- D. V. Titov and J. O. Liu, *Bioorg. Med. Chem.*, 2012, **20**, 1902-1909.
- E. Smith and I. Collins, *Future Med. Chem.*, 2015, **7**, 159-183.
- H. Guo and Z. Li, *Medchemcomm.*, 2017, **8**, 1585-1591.
- Z. Li, P. Hao, L. Li, C. Y. Tan, X. Cheng, G. Y. Chen, S. K. Sze, H. M. Shen and S. Q. Yao, *Angew. Chem., Int. Ed.*, 2013, **52**, 8551-8556.
- Z. Li, D. Wang, L. Li, S. Pan, Z. Na, C. Y. Tan and S. Q. Yao, *J. Am. Chem. Soc.*, 2014, **136**, 9990-9998.
- S. Kellner, S. Seidu-Larry, J. Burhenne, Y. Motorin and M. Helm, *Nucleic Acids Res.*, 2011, **39**, 7348-7360.
- S.-Y. Dai, D. Yang, *J. Am. Chem. Soc.*, 2020, **40**, 17156-17166.
- E. Păunescu, L. Louise, L. Jean, A. Romieu and P.-Y. Renard, *Dyes Pigm.*, 2011, **91**, 427-434.
- K. Chiba, M. Asanuma, M. Ishikawa, Y. Hashimoto, K. Dodo, M. Sodeoka and T. Yamaguchi, *Chem. Commun.*, 2017, **53**, 8751-8754.
- K. Chiba, Y. Hashimoto and T. Yamaguchi, *Chem. Pharm. Bull.*, 2017, **65**, 994-996.
- T. Tomohiro, A. Yamamoto, Y. Tatsumi and Y. Hatanaka, *Chem. Commun.*, 2013, **49**, 11551-11553.
- T. M. Ayele, S. D. Knutson, S. Ellipilli, H. Hwang and J. M. Heemstra, *Bioconjugate Chem.*, 2019, **30**, 1309-1313.
- Z. Li, L. Qian, L. Li, J. C. Bernhammer, H. V. Huynh, J. S. Lee and S. Q. Yao, *Angew. Chem., Int. Ed.*, 2016, **55**, 2002-2006.
- D. P. Murale, S. C. Hong, J. Yun, C. N. Yoon and J. S. Lee, *Chem. Commun.*, 2015, **51**, 6643-6646.
- G. Dorman, H. Nakamura, A. Pulsipher and G. D. Prestwich, *Chem. Rev.*, 2016, **116**, 15284-15398.
- D. I. Pattison, A. S. Rahmanto and M. J. Davies, *Photochem. Photobiol. Sci.*, 2012, **11**, 38-53.
- K. Renault, P.-Y. Renard and C. Sabot, *New J. Chem.*, 2017, **41**, 10432-10437.
- H. Mtiraoui, K. Renault, M. Sanselme, M. Msaddek, P. Y. Renard and C. Sabot, *Org. Biomol. Chem.*, 2017, **15**, 3060-3068.
- C. B. Mishra, S. Kumari, A. Angeli, S. M. Monti, M. Buonanno, M. Tiwari and C. T. Supuran, *J. Med. Chem.*, 2017, **60**, 2456-2469.
- J. Yang, J. Seckute, C. M. Cole and N. K. Devaraj, *Angew. Chem., Int. Ed.*, 2012, **51**, 7476-7479.
- X. Ji, K. Ji, V. Chittavong, R. E. Aghoghovbia, M. Zhu and B. Wang, *J. Org. Chem.*, 2017, **82**, 1471-1476.